Investigations of the Quorum Sensing Circuitry in *Burkholderia cenocepacia* H111

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Silja Inhülsen

aus

Hannover, Deutschland

Promotionskomitee
Prof. Dr. Leo Eberl (Vorsitz)
Prof. Dr. Kathrin Riedel
Prof. Dr. Jakob Pernthaler

Zürich, 2011

Investigations of the Quorum Sensing Circuitry in *Burkholderia cenocepacia* H111

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Silja Inhülsen

aus

Hannover, Deutschland

Promotionskomitee
Prof. Dr. Leo Eberl (Vorsitz)
Prof. Dr. Kathrin Riedel
Prof. Dr. Jakob Pernthaler

Zürich, 2011

Table of Contents

List of Figures and Tables ..... **i**
Zusammenfassung ..... **iii**
Summary ..... **iv**
Abbreviations ..... **v**

1 Introduction **1**

1.1 The genus Burkholderia 1
1.1.1 The *Burkholderia cepacia* complex (Bcc) 2
1.1.2 *Burkholderia cenocepacia*: a problematic opportunistic human pathogen 4
1.2 Cell-to-cell communication via quorum sensing 5
1.3 Genomic structure and quorum sensing circuitry in *B. cenocepacia* H111 6
1.4 Goals of this work 9

2 Material and Methods **11**

2.1 Material 11
2.1.1 Bacterial strains and plasmids 11
2.1.2 Media, chemicals and other material 12
2.1.3 Oligonucleotides 15

2.2 Molecular biological methods 16
2.2.1 Cultivation of bacterial strains and growth conditions 16
2.2.2 Manipulation of DNA 16
2.2.2.1 DNA preparation and characterization 16
2.2.2.2 Southern blot hybridization 17
2.2.2.3 Precipitation of DNA and RNA and quality analysis by gel electrophoresis 18
2.2.2.4 Recombinant DNA techniques 19
2.2.3 DNA sequence determination and database analysis 19
2.2.4 Allelic replacement and mutagenesis in *B. cenocepacia* H111 20
2.2.5 Construction of plasmid pJTR2, pBBR*rsaM* and pBBR*rsaM*_FS 21
2.2.6 Generation of plasmid pJBA89luxR$^-$ by partial digestion 21
2.2.7 Generation of plasmid pET-HisBclB 21
2.2.8 Transcriptional fusions to *lacZ* 21
2.2.9 RNA extraction and transcriptome analysis 22

2.3 Protein biochemical methods 23
2.3.1 Extraction of proteins and determination of protein amount 23
2.3.2 SDS-gel electrophoresis and protein visualization 24
2.3.3 Overproduction and purification of lectin BclB (BCAM0184) 25
2.3.4 Generation of BclB antibodies and Western immunoblot analysis 25
2.3.5 Detection of BclB by immunofluorescence microscopy 26
2.3.6 Protein extraction and analysis by iTRAQ 27

2.4 Phenotypical assays 30
2.4.1 Analyses of gene expression measuring β-galactosidase activity 30
2.4.2 Extraction and detection of acyl-homoserine lactone (AHL) molecules 31
2.4.2.1 Detection of AHL molecules employing sensor plasmids 31
2.4.2.2 Qualitative and quantitative detection of AHL molecules 32
2.4.2.3 Analyses employing reporter strains pJBA89 and pJBA89luxR$^-$ 33
2.4.2.4 Analyses of AHL production throughout the growth curve 33
2.4.2.5 Extraction of AHL molecules and detection by thin layer chromatography 33
2.4.3 Detection of extracellular protease activity 34
2.4.4 Extraction and detection of siderophores 34
2.4.4.1 Extraction of pyochelin 34
2.4.4.2 Extraction of ornibaction 34

2.4.4.3 Detection of siderophores on CAS plates and by TLC ... 35
2.4.5 Analysis of bacterial motility ... 36
2.4.6 Analysis of biofilm formation ... 36
2.4.6.1 Biofilm formation under static conditions in microtitre plates ... 36
2.4.6.2 Biofilm formation under dynamic conditions in artificial flow chambers ... 37
2.4.6.3 Visualization of dead bacteria by staining with propidiume iodide ... 38
2.4.7 Metabolic profiling employing BIOLOG microarrays ... 38
2.4.8 Pathogenicity model experiments ... 38
2.4.8.1 The *Caenorhabditis elegans* infection model ... 38
2.4.8.2 *Galleria mellonella* killing assay ... 40

3 Results ... 41

3.1 Project I: The *Burkholderia cenocepacia* H111 quorum sensing regulon: a comparative transcriptomic, proteomic and phenotypic analysis ... 41
3.1.1 Mapping the CepR regulon of *B. cenocepacia* H111 by transcriptomics ... 41
3.1.2 Identification of QS-regulated proteins in *B. cenocepacia* H111 ... 42
3.1.3 Correlation between transcriptomic and proteomic data ... 43
3.1.4 The H111 CepR-activated regulon identified by transcriptomics and proteomics ... 44
3.1.5 Overexpression of CepR leads to reduced expression of flagellar-associated genes ... 48
3.1.6 Quorum sensing appears to only slightly affect *B. cenocepacia* H111 metabolism ... 49

3.2 Project II: The role of the orphan LuxR homologue CepR2 in the transcriptional regulation of H111 genes and its effect on the quorum sensing circuitry ... 51
3.2.1 Construction and characterization of a *cepR2* mutant in H111 ... 52
3.2.2 CepR2 is involved in the production of pyochelin in *B. cenocepacia* H111 ... 53
3.2.3 CepR2 – an AHL-independent transcriptional regulator ... 55

3.3 Project III: Characterization of the BclACB lectins of *B. cenocepacia* H111, examination of their expression, subcellular localization and their role in biofilm formation ... 59
3.3.1 BclACB lectins are required for biofilm structural development ... 59
3.3.2 The Bcl orthologues are required for virulence in a *C. elegans* infection model ... 63
3.3.3 Expression of the *bclACB* operon is CepR-induced and dependent on CepR2 ... 64
3.3.4 Analyses of BclB expression by Western immunoblotting ... 65
3.3.5 Lectin BclB is associated with the bacterial surface ... 67

3.4 Project IV: Characterization of the RsaM regulator ... 69
3.4.1 RsaM affects the QS circuitry and encodes a protein of unknown function ... 69
3.4.2 Phenotypic characterization and transcriptome analyses of mutant R26 ... 70
3.4.3 Phenotypic characterization of mutant H111-rsaM and complementation ... 73
3.4.4 RsaM seems to modulate the activity of LuxR family transcriptional regulators ... 77
3.4.4.1 RsaM seems to influence the activity of CepR2 ... 77
3.4.4.2 RsaM seems to indirectly affect the transcription of CepR-regulated genes ... 79
3.4.4.3 RsaM seems to modulate protein activities of LuxR ... 81
3.4.5 RsaM negatively affects extracellular levels of AHL signal molecules ... 82

4 Discussion and Outlook ... 85

4.1. Quorum sensing in *B. cenocepacia* H111 ... 85
4.1.1. The RsaM regulator and production of AHL molecules ... 86
4.1.2. The orphan LuxR homologue CepR2 ... 89
4.2. The BclACB lectins ... 91

5 References ... 95
6 Appendix ... 104

Acknowledgements ... 144
Curriculum Vitae ... 145

List of Figures and Tables

Figures

Fig. 1. Phylogenetic tree of *Burkholderia complex* bacteria (Bcc) 3
Fig. 2. Schematic presentation of the *B. cenocepacia* H111 genomic structure and QS circuitry. 8
Fig. 3. System set-up of biofilms. 37
Fig. 4. Circular chart of QS-regulated proteins identified in two independent iTRAQ analyses. 43
Fig. 5. Overlap analysis of 3-fold differentially expressed genes and gene products. 44
Fig. 6. Predicted QS-regulated operons in *B. cenocepacia* H111 47
Fig. 7. Swimming activity of *B. cenocepacia* H111 wt, mutants and complemented strains..... 48
Fig. 8. Amino acid sequence alignment of CepR2 with LuxR-type transcriptional regulators..... 51
Fig. 9. Interruption of the *B. cenocepacia* cepR2 gene via single cross-over recombination events. 52
Fig. 10. CAS activity of *B. cenocepacia* strains. 53
Fig. 11. Production of pyochelin (A) and ornibactin (B) in *B. cenocepacia* strains. 54
Fig. 12. CepR2 controls the activity of the *pchR* promoter..... .55
Fig. 13. Deletion of *luxR* from plasmid pJBA89 by partial digestion with *Hind*III. 56
Fig. 14. CepR2 activates a P_{luxI}-*gfp* transcriptional fusion in an AHL-independent manner. 57
Fig. 15. Generation of the lectin deletion mutant H111-*bclACB.* 60
Fig. 16. Biofilm formation of the *B. cenocepacia* H111 wild type and mutants in polystyrene microtitre-dishes..... 61
Fig. 17. Biofilm structures of the *B. cenocepacia* H111 wild type and mutant H111-*bclACB*..... 62
Fig. 18. Hollow colonies in mutant H111-*bclACB* are not due to cell death ..... .63
Fig. 19. Killing of nematodes is reduced in *B. cenocepacia* mutant H111-*bclACB* in comparison to the wild type. 64
Fig. 20. Organisation of the *bclACB* genes on *B. cenocepacia* chromosome 2. 64
Fig. 21. Transcription of *bclA* is induced by CepR and CepR2. 65
Fig. 22. QS-dependent expression of lectin BclB. 66
Fig. 23. Binding of Alexa Fluor® 594-conjugated BclB antibodies to the cell surface of GFP-fluorescent *B. cenocepacia* H111. 68
Fig. 24. Genetic organisation of the *B. cenocepacia cepR* (BCAM1868), *rsaM* (BCAM1869) and *cepI* (BCAM1870) genes. 70
Fig. 25. Swarming activities of the *B. cenocepacia* H111 wt, mutants and complemented strains 74
Fig. 26. Biofilm formation of the *B. cenocepacia* H111 wt, QS mutant and complemented strains. 74
Fig. 27. Protease activities of the *B. cenocepacia* H111 wt, mutants and complemented strains. 75
Fig. 28. Killing of nematodes by the *B. cenocepacia* H111 wt, mutants and complemented strains. 75
Fig. 29. CAS activities of *B. cenocepacia* H111 wild type, mutants and complemented strains..... 76
Fig. 30. Detection of AHL production of the *B. cenocepacia* H111 parent and mutants via cross-streaking. 77
Fig. 31. Promoter activities of *pchR* are not reduced in mutant H111-rsaM..... .78
Fig. 32. Activities of the *cepI* promoter and the *aidA* promoter are dependent on RsaM. 79
Fig. 33. RsaM does not seem to stimulate the CepR-mediated activation of a P_{cepI}-*gfp* transcriptional fusion. 80
Fig. 34. RsaM seems to repress the LuxR-mediated activation of a P_{luxI}-*gfp* transcriptional fusion... 81
Fig. 35. Detection of AHL molecules by TLC and AHL sensor plasmid pAS-C8.. 82
Fig. 36. Analysis of AHL production along the bacterial growth curve. 83
Fig. 37. AHL production of *B. cenocepacia* H111 mutants and *cepI* complemented mutants harbouring plasmid pBBR1MCScepI. 84
Fig. 38. Hypothetical model of the CepIR QS network in *B. cenocepacia* H111. 85
Fig. 39. Amino acid sequence alignment of RsaM with homologous proteins.. 86

Fig. A1. Transcription of *aidA* is stringently regulated by CepR..... 104
Fig. A2. Metabolic profiling of *B. cenocepacia* H111 strains using BIOLOG microtiter plates..... 105
Fig. A3. Growth differences between the *B. cenocepacia* H111 wild type and mutant strains on minimal medium supplemented mit D-arabinose. 106

Tables

Table 1. Bacterial strains and plasmids used in this study 12
Table 2. Antibiotics and other supplements used in this study 14
Table 3. Oligonucleotides used in this study. 15
Table 4. Composition scheme for the preparation of SDS-gels. 24
Table 5. CepR-induced genes and proteins. 46
Table 6. Growth differences between the H111 wild type, the *cepR* mutant H111-R and the complemented mutant H111-R (*cepR*$^+$) 50
Table 7. Differentially regulated genes and ORFs in the transposon insertion mutant R26 72
Table 8. Expression of QS-activated functions in the H111 mutants H111-R, R26 and H111-rsaM in comparison to the wild type. 73

Table A1. Genes and ORFs, tRNA and intergenetic regions which are more than 2-fold differentially regulated in the H111-R transcriptome versus the H111 wild type 107
Table A2. Genes and ORFs, tRNA and intergenetic regions which are more than 2-fold differentially regulated in the H111-R (*cepR*$^+$) transcriptome versus the H111 wild type. 110
Table A3. QS-regulated proteins which are more than 1.5-fold differentially expressed in mutant H111-I and/or mutant H111-R 116

Zusammenfassung

Burkholderia cenocepacia ist ein Gram-negatives Bakterium, welches ubiquitär in der Umwelt vorkommt. Vor drei Jahrzehnten wurde *B. cenocepacia* als verursachender Erreger von schwerwiegenden Lungenentzündungen in Patienten mit zystischer Fibrose (CF) identifiziert. Die Infektionen waren von einer charakteristischen Bakteriämie gekennzeichnet und führten zum frühen Tod der CF-Kranken. Seither wurde *B. cenocepacia* als ein zunehmend problematisches opportunistisches Pathogen in immunsupprimierten Individuen wahrgenommen, welches die Notwendigkeit für die weitere Erforschung und Aufklärung von Virulenzfaktoren von *B. cenocepacia* begründet. Es wurde aufgezeigt, dass die Ausbildung von pathogenen Eigenschaften in *B. cenocepacia* entscheidend durch „Quorum sensing“ (QS) beeinflusst wird, welches vor allem duch das CepIR-Regulationssystem vermittelt wird, das die Expression bestimmter Gene in Abhängigkeit von der Populationsdichte kontrolliert.

In der vorliegenden Arbeit wurde das QS-Regulon von *B. cenocepacia* H111 entschlüsselt und detalliert analysiert. Die Identifizierung von CepIR-regulierten Funktionen gelang dabei durch die Implementierung einer kombinierten transkriptionellen, proteomischen und phänotypischen Herangehensweise. Die Ergebnisse dieser Analyse liefern neue Erkenntnisse über im Vorfeld identifizierte QS-regulierte Phänotypen einschliesslich der Bildung von Biofilmen und der Pathogenität, und offenbaren wichtige Einblicke in den QS-Regulationskreislauf von *B. cenocepacia* H111.

Im zweiten Teil der Arbeit wurden die Produkte von drei QS-aktivierten Gen-Loci, welche in dieser Ausarbeitung identifiziert wurden, funktionell charakterisiert. Diese beinhalteten den „verwaisten“ LuxR-homologen Transkriptionsregulator CepR2, die Lektine BclACB und BCAM1869, welches von einem Gen kodiert wird, das in der intergenetischen Region zwischen den *cepR* und *cepI* Genen lokalisiert ist. Die Ergebnisse dieser Arbeit zeigten, dass CepR2 ein AHL-unabhängiger Transkriptionsregulator ist. Desweiteren wurde nachgewiesen, dass CepR2 die Expression der Siderophore Pyochelin stimuliert und dabei als Downstream-Regulator im QS-Netzwerk agiert. In der vorliegenden Arbeit wurde die subzelluläre Lokalisierung des BclB Lektins determiniert und die Bedeutung der BclACB-Proteine bei der strukturellen Entwicklung von Biofilmen in H111 untersucht.

Zudem wurde ein neuer Regulator, BCAM1869, identifiziert, welcher die Produktion von AHL-Molekülen in *B. cenocepacia* H111 hemmt. Hinzukommend scheint BCAM1869 die Aktivität von LuxR-ähnlichen Transkriptionsregulatoren zu beeinträchtigen, einschliesslich des *Vibrio fischeri* LuxR-Regulators. Die Ergebnisse dieser Arbeit weisen auch darauf hin, dass BCAM1869 die Aktivität von CepR2 in *B. cenocepacia* H111 positiv beeinflusst.

Summary

Burkholderia cenocepacia is a Gram-negative bacterium which is ubiquitously found in the environment. Three decades ago, *B. cenocepacia* was identified as the causative agent of severe pulmonary infections in patients with cystic fibrosis (CF). The disease was accompanied by a characteristic bacteraemia that resulted in the early death of CF individuals. Since then, *B. cenocepacia* has emerged as a problematic opportunistic pathogen in patients with immunodeficiency disorders demonstrating the need for further exploration and elucidation of factors which contribute to *B. cenocepacia* virulence. It has become clear that the expression of pathogenic traits in *B. cenocepacia* is at least partly controlled by quorum sensing (QS), a cell density-dependent regulatory process.

In the present thesis, the QS regulon of *B. cenocepacia* H111, which is comprised by the *cepIR* genes, was mapped and studied in detail. CepIR-regulated functions were identified by a combined transcriptomic, proteomic and phenotypic approach. The results of this analysis provided new insights into the molecular mechanisms of previously identified QS-regulated phenotypes, including biofilm formation, and pathogenicity, and revealed novel information of the complex QS circuitry operating in *B. cenocepacia* H111.

In the second part of this thesis, the products of three QS-activated loci, which were identified in our mapping study, were further characterized. These included the orphan LuxR homologous transcriptional regulator CepR2, the BclACB lectins, and BCAM1869, which is encoded by a gene located within the intergenetic region of the *cepR* and *cepI* genes. The results of this work identified CepR2 as an AHL-independent transcriptional regulator. Moreover, CepR2 was shown to stimulate expression of the siderophore pyochelin, while acting as a downstream regulator of the H111 QS network. In the present thesis, the subcellular localization of lectin BclB was determined, and the importance of the BclACB proteins for the structural development of H111 biofilms was investigated. The results obtained suggest that BCAM1869 fine-tunes QS regulation: BCAM1869 represses the producton of AHL signal molecules in *B. cenocepacia* H111. Furthermore, BCAM1869 seems to interfere with the activity of LuxR-like transcriptional regulators, including the *Vibrio fischeri* LuxR protein. The results of this work also suggest that BCAM1869 positively influences the activity of CepR2 in *B. cenocepacia* H111.

Abbreviations

AHL	*N*-acyl homoserine lactone
Bcc	*Burkholderia cepacia* complex
BCESM	*Burkholderia cepacia* epidemic strain marker
bp	base pair
BSA	bovine serum albumine
°C	degree Celsius
cDNA	complementary DNA
CF	cystic fibrosis
CGD	chronic granulomatous disease
CLSM	confocal laser scanning microscopy
C6-HSL	*N*-hexanoyl homoserine lactone
C8-HSL	*N*-octanoyl homoserine lactone
C-source	carbon source
cm	centimeters
dH_20	deionised water
ddH_20	Milli-Q water
DMF	dimethyl fumarate
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxinucleotide triphosphate
EDTA	ethylendiamintetraacetate
et al.	et alii
EtOH	ethanol
Fig.	figure
g	gram
GFP	green fluorescent protein
h	hour
HDTMA	hexadecyltrimethylammonium bromide
HSL	homoserine lactone
Hz	hertz
IPTG	isopropyl β-D-1-thiogalactopyranoside
iTRAQ	isobaric tag for relative and absolute quantitaion
kb	kilobases
kDa	kilodalton
kV	kilovolt
l	liter
LB-medium	Luria Bertani-medium
LC-MS/MS	liquid chromatography-tandem mass spectrometry
M	molar
mA	milliampere
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
MCS	multiple cloning site
µF	microfarad
µg	microgram
mg	milligram
min	minute
µl	microliter
ml	milliliter
µm	micrometer
µM	micromolar
mM	millimolar
MQ	Milli-Q water
MS	mass spectrometry
mw	molecular weight
N	normal
NBT/BCIP	nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate
NCBI	National Centre for Biotechnology Information
ng	nanogramm

NGM	nematode growth medium
nm	nanometer
nM	nanomolar
nt	nucleotide
OD	optical density
ONPG	O-nitrophenyl-β-D-galactopyranoside
ORF	open reading frame
OHL	*N*-octanoyl-homoserine lactone (C8-HSL)
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pmol	picomole
PM	phenotypic microarray
QS	quorum sensing
RBS	ribosome binding site
RFU	relative fluorescence unit
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
s	seconds
SD	standard deviation
SDS	sodium dodecyl sulphate
sRNA	small ribonucleic acid
TAE	Tris/acetic acid/EDTA
TCA	trichloroacetic acid
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)-aminomethan
U	unit
UV	ultraviolet
V	Volt
v/v	volume per volume
w/v	weight per volume
wt	wild type
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

1 Introduction

Burkholderia cenocepacia is a Gram-negative bacterium which is prevalent in soil habitats (Miller *et al.*, 2002). About three decades ago, *B. cenocepacia* was identified as a causative agent of severe pulmonary infections in patients with cystic fibrosis (CF), the most common lethal inherited disease among the Caucasian population (Tummler & Kiewitz, 1999). The infections were accompanied by a chronic pneumonia, sepsis and a characteristic bacteraemia which resulted in early death of the CF individuals (Isles *et al.*, 1984). Since then, *B. cenocepacia* has emerged as a problematic opportunistic pathogen, which can also cause infections in patients with chronic granulomatous disease (CGD), and is responsible for several recent nosocomial outbreaks (Speert, 2001, Mahenthiralingam *et al.*, 2005). Whereas the identity of many *B. cenocepacia* virulence factors has not yet been identified, it is known that the expression of pathogenic traits is affected by quorum sensing (QS), a cell density-dependent regulatory process (Sokol *et al.*, 2003, Köthe *et al.*, 2003). Infections with *B. cenocepacia* are highly transmissable and have become particularly problematic with increasing numbers of strains that have acquired resistences to clinically important antibiotics. For these reasons there is a need for the identification and characterization of *B. cenocepacia* virulence factors.

1.1 The genus Burkholderia

The genus *Burkholderia* comprises more then 40 species, which occupy a broad array of ecological niches. Most of the species are characterized by a remarkable metabolical versatility which allows the adaption to varying environmental conditions including nutrient limitation or the presence of antibiotics and toxic compounds (Coenye & Vandamme, 2003). Traditionally, *Burkholderia* species were identified as plant pathogens and soil bacteria with two important exceptions, *Burkholderia mallei* and *Burkholderia pseudomallei*, which are primary pathogens for humans and animals. *Burkholderia* species have been moreover isolated throughout the environment including freshwater or fungal mycelia (Coenye & Vandamme, 2007) and are also encountered in human clinical sources where they are universal contaminants of cosmetics and other pharmaceutical solutions (Jimenez, 2001). The range of interactions between *Burkholderia* species and their environments or hosts is complex, and often contradictory. Their host range can vary dramatically, and the type of interaction may be that of a pathogen, but it can also be beneficial, dependening on the host. Thus, some species of the genus *Burkholderiae* exist as free-living cells while others engage in endosymbiotic relationships with fungi or plants (Coenye & Vandamme, 2007).

Burkholderia kirkii is living inside plant leaf galls (Van Oevelen *et al.*, 2002) and *B. pseudomonallei* was reported to proliferate within macrophages (Jones *et al.*, 1996). The exact mechanism by which the bacteria survive and persist within these hosts is not yet understood. Within the genus *Burkholderia* a group of species known as the *Burkholderia cepacia* complex (Bcc) attracted particular interest, mostly because of their recognition as important opportunistic pathogens for CF patients.

1.1.1 The *Burkholderia cepacia* complex (Bcc)

The *Burkholderia cepacia* complex (Bcc) is a collection of genetically distinct but phenotypically similar *Burkholderia* which currently comprise 17 validly species (Vanlaere *et al.*, 2008a). Bcc species share a high degree of 16S rRNA (98 - 100%) and *recA* (94 – 95%) sequence similarity but only moderate levels of DNA-DNA hybridization (30 – 50%) (Vandamme *et al.*, 1997, Vandamme *et al.*, 2003, Coenye *et al.*, 2001, Vermis *et al.*, 2004). Bcc species are (Fig. 1): *Burkholderia cepacia*, *Burkholderia multivorans*, *Burkholderia cenocepacia*, *Burkholderia stabilis*, *Burkholderia vietnamiensis*, *Burkholderia dolosa*, *Burkholderia ambifaria*, *Burkholderia anthina*, *Burkholderia pyrrocinia*, *Burkholderia ubonensis*, *Burkholderia latens*, *Burkholderia diffusa*, *Burkholderia arboris*, *Burkholderia seminalis*, *Burkholderia metallica*, *Burkholderia lata*, and *Burkholderia contaminans*.

Reflecting their extraordinary metabolic versatility, Bcc species inhabit a wide variety of environmental niches, including soil, water, rhizospheres, industrial settings, and cosmetics (Mahenthiralingam *et al.*, 2008, Coenye & Vandamme, 2003, Mahenthiralingam *et al*., 2005). Some Bcc strains are moreover capable of degrading organic compounds as for instance herbicides (Stanier *et al.*, 1966), pesticides (Holmes *et al.*, 1998), and trichloroethylene (Folsom *et al.*, 1990), therefore bearing a great potential as bioremediation agents. Other Bcc species, as *B. ambifaria*, are recognized for their substantial beneficial properties in the agricultural industry where they are used as biological control agent (Holmes *et al*., 1998) or biopesticides in the production of crops (Ciccillo *et al.*, 2002). Bcc strains can thereby colonize the rhizosphere of several crops including maize, rice, corn, pea, and sunflower which results in a significantly increase in the crop yield, an effect referred to as plant growth promotion (Parke, 1991, Tran Van, 2000).

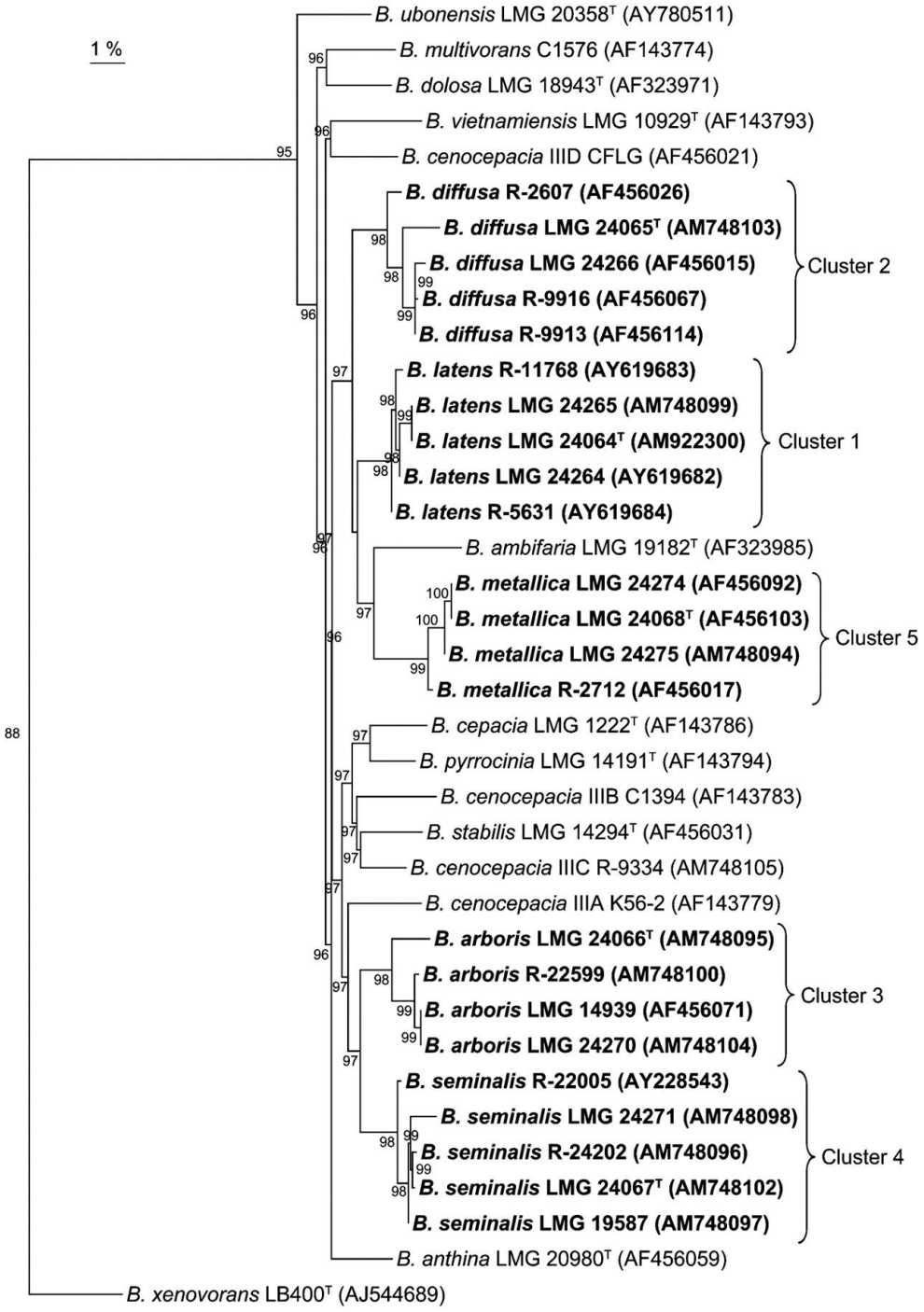


Fig. 1. Phylogenetic tree of *Burkholderia complex* bacteria (Bcc) and five novel Bcc clusters derived from the analysis of *recA* gene sequences. Different *B. cenocepacia recA* lineages are designated IIIA, IIIB, IIIC and IIID. The tree was constructed using the neighbour-joining method (Jukes & Cantor parameter). Accession numbers are given in parentheses. Bootstrap values (>70 %) are shown for 1000 replicates. *B. xenovorans* LB400^T was used as the out group. Scale bar indicates 1% sequence dissimilarity (Vanlaere *et al.*, 2008b).

Importantly, besides their biotechnolocial application, Bcc strains also bear a high pathogenic potential. Even though *B. multivorans* and *B. cenocepacia* are most frequently isolated from CF patients, all Bcc species have also been isolated from clinical samples belonging to individuals with CF (Lessie *et al.*, 1996, Parke & Gurian-Sherman, 2001, Vanlaere *et al.*, 2009). The number of human infections caused by Bcc strains has markedly increased in the past two decades (Mahenthiralingam *et al*., 2005, Mahenthiralingam *et al*., 2008) and Bcc strains have moreover caused several outbreaks within and between regional center of CF patients (Saiman & Siegel, 2004).

The need for a taxonomic reclassification of the original as "*Pseudomonas cepacia*" or "*Burkholderia cepacia*" named species into distinct genomic (Bcc) species emerged when traditional molecular identification approaches including growth on selective media, commercial biochemical microtest systems, whole cell fatty acid analysis and several PCR-based techniques (Bevivino *et al.*, 1994, Tabacchioni *et al.*, 1995, Coenye & Vandamme, 2007) were demonstrated to be insufficient for a proper classification. Taxonomic studies were initiated to determine if clinical *Burkholderia* isolates also occur in the environment. The taxonomic classification into Bcc complex species was implemented by Vandamme and collegues in 1997 (Vandamme *et al*., 1997). They were able to demonstrate that "*B. cepacia"* isolates from CF patients, other human clinical samples, and the environment belonged to at least five distinct genomic species (Coenye & Vandamme, 2007). Employing multilocus sequence typing (Baldwin *et al.*, 2007) it was later shown that one-fifth of the clinical isolates of Bcc species were indistinguishable from environmental isolates. As this finding suggests that Bcc infections can be acquired by strains normally living in the environment, the commercial use of Bcc species in the agricultural industry has been stopped.

1.1.2 *Burkholderia cenocepacia*: a problematic opportunistic human pathogen

Burkholderia cenocepacia, a *Burkholderia cepacia* complex species (Bcc, see 1.1.1), is together with *B. multivorans* the most clinically important human pathogen which can cause life-threatening lung infections in immunocompromised individuals. It has been suggested that *B. cenocepacia* can replace *B. multivorans* during chronic CF infections (Mahenthiralingam *et al.*, 2002). Individuals with CF are particularly vulnerable to acquire chronic airway infections because they hold a highly viscous mucus in their lung, which impairs a ciliary clearance and often favours the colonization with opportunistic bacterial pathogens, particularly *Staphylococcus aureus* and *Haemophilus influenza* and, at a later point of the infection, *P. aeruginosa* (Tummler & Kiewitz, 1999, Eberl & Tummler, 2004, Ratjen &

Doring, 2003). Bcc strains are often acquired by CF patients in the late process of the disease when they are already chronically colonised with *P. aeruginosa*. The clinical outcome of this co-infection is variable and unpredictable and can range from an asymptomatic carriage to a fulminant and fatal pneumonia, the so-called 'cepacia syndrome' (Isles *et al*., 1984). In addition to acquisition from the environment *B. cenocepacia* has the greatest risk of patient-to-patient transmission (Mahenthiralingam *et al.*, 2001, LiPuma *et al.*, 1988, Coenye & LiPuma, 2002, Chen *et al.*, 2001).

An important role in the infection process appears to be the formation of biofilms on the epithelial cell surface of the lung. It has been suggested that the attachment of Bcc strains is facilitated by *P. aeruginosa* exoproducts which may result in the development of mixed biofilms (Saiman *et al.*, 1990). The formation of a biofilm is particularly problematic in clinical settings because bacteria living in biofilms can withstand host immune responses and exhibit an increased resistance towards antibiotics.

The pathogenic mechanisms and virulence determinants of Bcc species, including *B. cenocepacia*, are still poorly understood. It has been shown that the production of extracellular factors such as siderophores, proteases, and lipases contribute to the pathogenicity of *B. cenocepacia* (Lewenza *et al*. 1999; Lewenza and Sokol, 2001). The expression of *B. cenocepacia* pathogenic traits has been shown to be at least in part regulated by the CepIR quorum sensing network (see 1.2 and 1.3).

The model strain used in the Zurich laboratory is *B. cenocepacia* H111, an isolate from a CF patient in Hannover (Geisenberger *et al.*, 2000), that is very closely related to the sequenced strain *B. cenocepacia* J2315 (Romling *et al.*, 1994, Gotschlich *et al.*, 2001). However, in contrast to J2315, *B. cenocepacia* H111 does not belong to the epidemic ET12 lineage (see 1.2.3).

1.2 Cell-to-cell communication via quorum sensing

The realization that bacteria do not behave as solitary, individual cells, as once thought, but rather communicate with each other, certainly accounts for one of the most fascinating biological discoveries in the last decades. The cellular concept standing behind this insight was originally discovered unraveling the phenomenon of bioluminsense in the Gram-negative marine organism *Vibrio fischeri* (Nealson *et al.*, 1970) and was initially referred to as autoinduction (Fuqua *et al.*, 1994) to emphazise that this phenomenon depends on a minimal population size, i.e. a critical number or quorum (latin: "of whom") of individual cells to trigger gene expression. This phenomenon is commonly refered to as "quorum sensing" (QS).

QS can be defined as a generic regulatory process used by bacteria to sense and response to the densitiy of bacterial populations in a coordinated way (Fuqua *et al*., 1994). As QS allows populations of bacteria to collectively control gene expression and thus synchronize group behaviour, it is seen as a system of cell-to-cell communication, which enables the coordination of bacterial cells similar to that of multicellular organisms. Processes controlled by QS are therefore typically ones that are unproductive unless many bacteria act together, as functions involved in pathogenicity, antibiotic production, or surface colonization. QS systems have been identified in Gram-positive and more than 80 species of Gram-negative bacteria, in which they regulate a variety of functions including, for instance, the conjugative transfer of plasmids (Williams *et al.,* 2007). Cell-to-cell communication via QS relies on small signal molecules, of which various have been described. The two most thoroughly investigated classes are the *N*-acyl-homoserine lactones (AHL), which are produced by many Gram-negative bacteria, and small peptides, which are utilized by many Gram-positive species (Fuqua *et al.*, 1996, Whitehead *et al.*, 2001).

1.3 Genomic structure and quorum sensing circuitry in *B. cenocepacia* H111

A common trait of Bcc bacteria is the presence of unusual large genomes which are moreover composed of multiple replicons and share high amount of insertion sequences (Lessie *et al*., 1996). All characterized Bcc species have a minimum of three large chromosomal replicons and an average genome size of 7.5 Mb encoding roughly over 7000 genes (Mahenthiralingam *et al*., 2005). *B. cenocepacia* H111 is a very closely relative to the sequenced strain *B. cenocepacia* J2315, which genome consists of three circular chromosomes of 3,870,082 bp, 3,217,062 bp, and 875,977 bp and a plasmid of 92,661 bp (Holden *et al.*, 2009). In costrast to J2315, and to all other strains belonging to the epidemic ET12 lineage, the genome of *B. cenocepacia* H111 does not comprise a plasmid. Moreover ET12 lineage strains encode a 31.7 kb-large pathogenicity island on chromosome 2 (Baldwin *et al.*, 2004, Malott *et al.*, 2005), which is also absent in the genome of H111. This island encodes an additional QS system, the CciIR system (Malott *et al*., 2005). *B. cenocepacia* H111 only contains the CepIR system, which is believed to be the ancestral AHL-based QS system in the genus *Burkholderia* (Fig. 2). CepIR homologous systems have been identified in all Bcc species (Gotschlich *et al*., 2001).

The CepIR system consists of the transcriptional regulator CepR and the acyl-homoserine lactone (AHL) synthase CepI, which directs the synthesis of the two AHLs *N*-octanoyl-homoserine lactone (C8-HSL) and, as a minor product, *N*-hexanoyl-homoserine lactone (C6-

HSL) (Gotschlich *et al*., 2001). At low population densities a basal level of AHL molecules is synthesized. With increasing cell numbers the concentration of the signal molecules then increases and molecules diffuse into the extracellular milieu. On reaching a critical threshold concentration, the signal molecules bind to its cognate receptor CepR, which, in this complex, then activates or represses the transcription of target genes. As demonstrated by Lewenza and coworkers (Lewenza *et al.*, 1999) the transcription of *cepI* is autoregulated, in that the complex of C8-HSL/CepR stimulates the transcription of *cepI*, presumably by binding to a DNA motiv within *cepI* promoter regions. This 20 bp imperfect palindrome, termed "*cep* box", resembles the *lux* box motif described in *Vibrio fischeri* (Devine *et al.*, 1989, Lewenza *et al*., 1999).

Functions positively regulated by the CepIR system include the production of extracellular proteases and chitinases, the production of the siderophore pyochelin and the synthesis of the nematocidal protein AidA (Huber *et al.*, 2001, Huber *et al.*, 2002, Malott *et al.*, 2009, Huber *et al.*, 2004, Köthe *et al*., 2003). Previous work has also demonstrated that both, the ability to swarm, and the late stages of biofilm development in *B. cenocepacia* H111 are QS-regulated (Huber *et al*., 2002). The synthesis of the siderophore ornibactin has by contrast been shown to be repressed by QS in strains *B. cenocepacia* K56-2 (Lewenza & Sokol, 2001) and *B. cenocepacia* H111 (Malott *et al*., 2009).

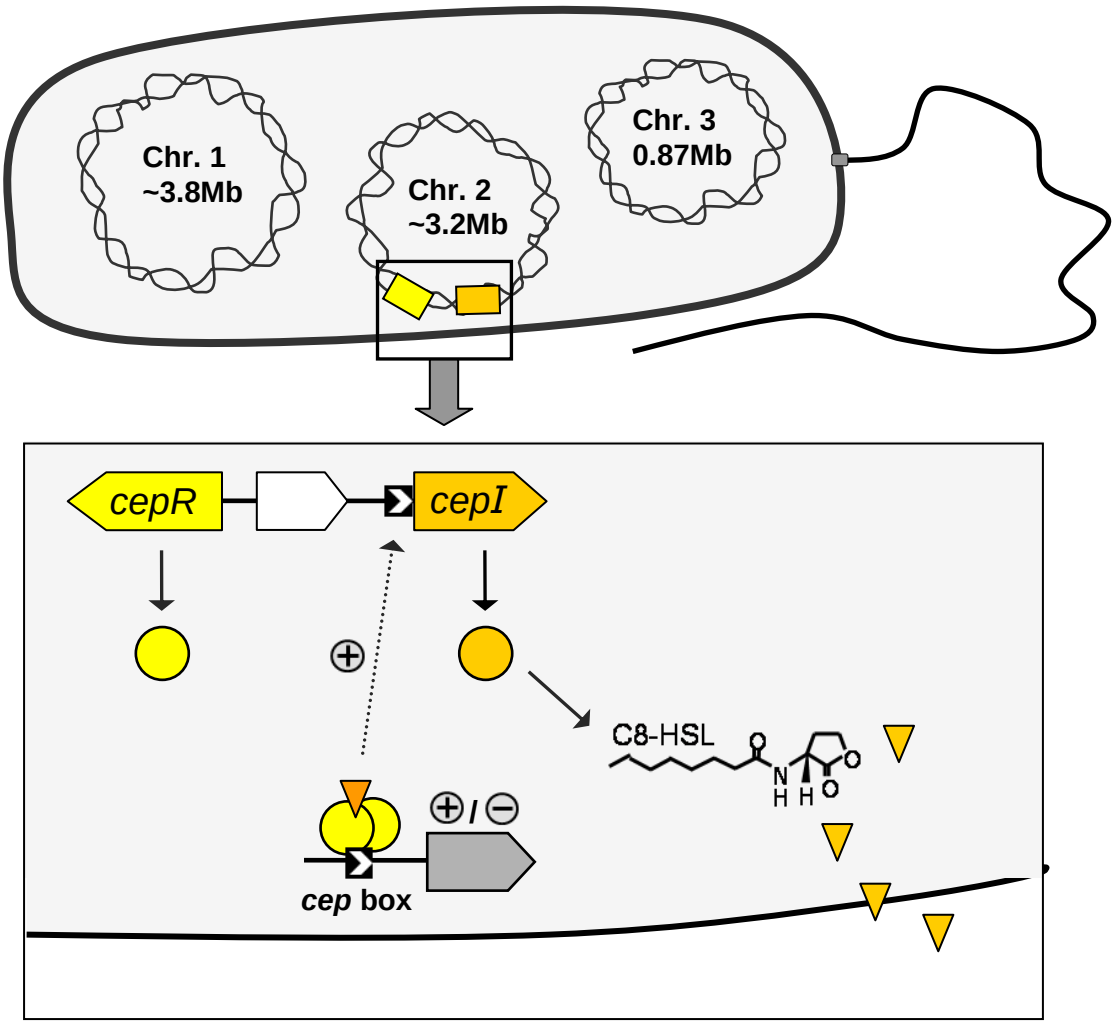


Fig. 2. Schematic presentation of the *B. cenocepacia* H111 genomic structure and QS circuitry. The illustration shows the genome structure of *B. cenocepacia* H111 including the three chromosomal replicons (Chr.1, Chr. 2, and Chr. 3), (Mahenthiralingam *et al*., 2005). The CepIR system, which is present in all Bcc species, is encoded on chromosome 2 and comprises the transcriptional regulator CepR and the acyl-homoserine lactone (AHL) synthase CepI, which directs the synthesis of the diffusible signal molecules N-octanoyl homoserine lactone (C8-HSL) and, to minor amounts *N*-hexanoyl homoserine lactone (C6-HSL) (Gotschlich *et al*., 2001). When a critical population density has been attained, the CepR protein binds in complex with C8-HSL to the promoter of target genes and thereby induces or represses the transcription of these genes. Promoter binding generally occurs at a specific DNA element, the so-called "*cep* box" (for sequence see Fig. 6), (Lewenza *et al*., 1999). The expression of *cepI* has been shown to be positively autoregulated by QS (dashed line, Lewenza *et al*., 1999). Functions which are induced by the *B. cenocepacia* H111 CepIR system include the production of extracellular proteases, chitinases, and the siderophore pyochelin, swarming motility, biofilm formation, and the expression of the nematocidal protein AidA (Huber *et al*., 2004, Huber *et al*., 2001, Huber *et al*., 2002, Köthe *et al*., 2003, Malott *et al*., 2009). The production of ornibactin is repressed by the CepIR system (Malott *et al*., 2009).

1.4 Goals of this work

Project I: The *Burkholderia cenocepacia* H111 quorum sensing regulon: a comparative transcriptomic, proteomic and phenotypic analysis

Burkholderia cenocepacia H111 utilizes the CepIR quorum sensing (QS) system to express various functions in a population density-dependent manner. It has become clear that the expression of various pathogenic traits, including the production of extracellular proteases or siderophoes, the ability to swarm, and the formation of a biofilm, is QS-regulated in the large majority of *B. cenocepacia* species (Wopperer *et al.*, 2006). Some of these phenotypes have been linked to QS-dependent expression of specific genes, as demonstrated for the nematocidal protein AidA (Huber *et al.*, 2004). Many of the QS-regulated genes or proteins, which account for the pathogenic potential of H111, including those required for the formation of a biofilm, have not been identified yet. The aim of this project was to definde the *B. cenocepacia* H111 QS regulon, to understand the impact of cell-to-cell communication on the expression of pathogenic traits and factors required for biofilm formation. For that purpose, the CepIR regulon was mapped using a combined transcriptomic, proteomic and phenotypic approach: transcriptomic profiling employed Agilent *B. cenocepacia* oligonucleotide microarrays, the proteome analysis employed iTRAQ (isobaric tag for relative and absolute quantitation), and the phenotypical characterization used BIOLOG microarrays.

Project II: The role of the orphan LuxR homologue CepR2 in the transcriptional regulation of H111 genes and its effect on the quorum sensing circuitry

A previous screening of a *Burkholderia cepacia* transposon library identified a LuxR homologous protein, which was designated CepR2. CepR2 is an "orphaned" LuxR transcriptional regulator, since the *cepR2* gene (BCAM0188) was found to be located without a proximal *luxI* family gene. This fact, together with findings made in a previous study (A. Steidle, diploma thesis, TU Munich, 1999) suggested that CepR2 might regulate the transcription of genes independent of the presence of AHL molecules. This project was initiated to understand the involvement of CepR2 in the QS network of *B. cenocepacia* H111 and to investigate if this regulator plays a role in the expression of virulence factors. It was also of particular interest to provide unambiguous evidence that CepR2 functions in an AHL-independent manner.

Project III: Characterization of the BclACB lectins of *B. cenocepacia* H111, examination of their expression, subcellular localization and their role in biofilm formation

The *B. cenocepacia* proteins BclA (BCAM0186), BclC (BCAM0185) and BclB (BCAM0184) belong to the PA-IIL family of lectins (Lameignere *et al.*, 2008), which are commonly thought to play important roles in processes that require cell adhesion or host recognition or defence mechanisms (Tielker *et al.*, 2005, Imberty *et al.*, 2005, Sudakevitz *et al.*, 2002, Zinger-Yosovich *et al.*, 2006, Imberty & Varrot, 2008). Previous studies focused on the analysis of the protein structures of lectin BclA (Lameignere *et al*., 2008, Lameignere *et al.*, 2010) and lectin BclC (Sulak *et al.*, 2010), and described the binding affinity of these lectins to carbohydrate ligands. The aim of this project was to elucidate the function of the Bcl proteins in *B. cenocepacia* H111 biofilm formation. Furthermore, it was also investigated, whether the Bcl proteins are, like other PA-IIL family proteins, associated with the bacterial cell surface. Finally, the impact of QS on the expression of BclACB was examined.

Project IV: Characterization of the RsaM regulator

In a previous study, a *B. cenocepacia* H111 transposon library (B. Huber *et al.*, 2002) was screened for mutants attenuated in *C. elegans* pathogenicity (M. Köthe, 2003, F. Feldmann, 2004). One mutant was of particular interest, because the strain not only exhibited a reduced virulence but was also found to produce increased levels of extracellular AHL signal molecules, as observed in this thesis. Sequences analyses revealed that the transponson had inserted in the intergenetic region of coding sequences belonging to the *cepR* and *cepI* genes, upstream of the ORF BCAM1869, which is predicted to encode a functionally unknown hypothetical protein (Wopperer, 2008, Dissertation, University of Zurich). The initial task of this project was to verify that BCAM1869, referred to as RsaM, encodes a protein. To determine the cellular role of BCAM1869 the transposon insertion mutant and a defined H111-rsaM mutant were phenotypically characterized. These investigations revealed that BCAM1869 exhibits a regulatory effect on the CepIR system as well as on the transcriptional regulators CepR and the orphan LuxR homologue CepR2. Finally, the impact of BCAM1869 on other LuxR-like proteins, including the LuxR transcriptional regulator, was examined.

2 Material and Methods

2.1 Material

2.1.1 Bacterial strains and plasmids

The bacterial strains and plamids used in this study are listed below.

Strain or DNA	Genotype or description	Source or reference
Strains		
Escherichia coli		
BL21 (DE3)	F$^-$ *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3)	(Weiner MP, 1994), Novagen
DH5α	F$^-$ Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) *recA1 endA gyrA96 thi-1 hsdR17 supE44 relAl deoR(U169)*	(Hanahan, 1983), Invitrogen
HB101 (pRK600)	F$^-$ *supE44 hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 recA thi pro leu hsdR- M+ Sm^r;*	(Boyer & Roulland-Dussoix, 1969)
MM294 (pRK2013)	F$^-$ *endA1 hsdR17 supE44*(AS) *rfbD1 spoT1 thi-1*; RK2 derivative, mob+ tra+ ori ColE1; Km^r	(Meselson & Yuan, 1968) (Figurski & Helinski, 1979)
SY327λpir	F$^-$ *araD* Δ(*lac-pro*) *argE* (Am) Rif^r *nalA recA56 λpir*	(Miller & Mekalanos, 1988)
TOP10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lacZ*_M15 Δ*lacX74 deoR recA1 araD139* Δ*(ara-leu)7697 galU galK rpsL(Str) endA1 nupG*	Invitrogen
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F´ proAB lacIq ZΔM15 Tn10 (Tcr)]*	Stratagene
MT102 (pSB403)	*luxRI*::*luxCDABE* transcriptional fusion, bioluminescent AHL sensor, Tc^r	(Winson *et al.*, 1998)
OP50	food source strain for *C. elegans*, uracil auxotrophic	(Brenner, 1974)
Pseudomonas putida		
F117	IsoF, mutation in *ppuI*, (AHL-negativ)	(Steidle *et al.*, 2001)
F117 (pAS-C8)	F117 with pBBR1MCS-5 carrying P_{cepI}::*gfp*(ASV) P_{lac}::*cepR;* Gm^r	(Riedel *et al.*, 2001)
Chromobacterium violaceum		
CV026	ATCC 31532 derivative, *cviI*::Tn5 *xylE*; Km^r, Sm^r	(McClean *et al.*, 1997)
Burkholderia cenocepacia		
H111	CF isolate from Germany, genomovar III	(Romling *et al*., 1994) (Gotschlich *et al*., 2001)
K56-2	CF isolate, BCESM+, *cblA*+	(Mahenthiralingam *et al.*, 2000)
H111-I	*cepI*::km derivative of H111; Km^r	Huber *et al.*, 2001
H111-I/R	marker less deletion mutant of *cepI*, *rsaM* and *cepR*, genotype: cepI/R::FRT	A. Carlier
H111-R	*cepR*::km mutant of H111; Km^r	Huber *et al.*, 2001
H111-R2	*cepR2*::pEX18Gm mutant of H111; Gm^r	This study
H111-R/R2	*cepR2*::pEX18Gm mutant of H111-R; Km^r, Gm^r	This study
H111-*bclABC*	*bclA-C*::km mutant of H111; Km^r	This study
H111-rsaM	*rsaM*::km mutant of H111; Km^r (mutant H111-V)	A. Carlier
Plasmids		
pASS2.10	*Sac*I-fragment of subclone S2, containing the *B. cenocepacia cepR2* pBBR1MCS2 background, Kan^r	A. Steidle, 2002
pAH1B.1	*Eco*RI-fragment of *B. cepacia*, clinical isolate, Nr. 767, puC18 background, Amp^r	A. Steidle, 2002
pAUC40	suicide vector ; Sm^r, Cm^r	(Carlier *et al.*, 2009)
pAUC40-bcl	pAUC40 containing the pKD4 kanamycin cassette and flanking regions of the *B. cenocepacia* H111 *bc*l operon (*bclA-C*)	This study

pBAH8	pBBR1MCS-5 containing PA1/04/03-*gfp* mut3-To-T1; Gm^r	Huber *et al.*, 2002
pBAH27	pBBR1MCS-5 containing the *cepR* gene of *B.* ceno*cepacia* H111, Gm^r	Huber *et al*., 2001
pBBR1MCS	broad host-range cloning vector; Cm^r	(Kovach *et al.*, 1994)
pBBR1MCS-2	broad host-range cloning vector; Km^r	(Kovach *et al.*, 1995)
pBBR1MCS-5	broad host-range cloning vector; Gm^r	(Kovach *et al*., 1995)
pBBR-cepR	pBBR1MCS containing the *cepR* gene of *B. cenocepacia* H111; Cm^r	S. Schmidt
pBBR*rsaM*	pBBR1MCS containing the *rsaM* gene of *B. cenocepacia* H111; Cm^r	This study
pBBR*rsaM*_FS	pBBR1MCS containing a frameshifted *rsaM* ORF of H111; Cm^r	This study
pCR2.1	cloning vector for PCR products; Amp^r, Km^r	Invitrogen
pDONR221	cloning vector; Km^r	Invitrogen
pEX18Gm	*oriT*$^+$ *sacB*$^+$*;* pUC18 MCS, gene replacement vector; Gm^r	(Hoang *et al*., 1998)
pEX19Gm	*oriT*$^+$, *sacB*$^+$, pUC19 MCS, gene replacement vector; Gm^r	(Hoang *et al*., 1998)
pEX18Gm-*R2*	Gm^r; *oriT*$^+$ *sacB*$^+$; Genaustauschvektor für H111-R2 und H111-R/R2	This study
pET28a	expression vector, T7 promoter; Km^r	Novagen
pJBA89	pUC18Not-*luxR*-P_{luxI}-RBSII-*gfp*(ASV)-T0-T1; Ap^r	(Andersen *et al.*, 1998)
pJBA89*luxR*$^-$	Apr, pUC18Not-P_{luxI}-RBSII-*gfp*(ASV)-T0-T1; Ap^r	This study
pET-HisBclB	pET28a derivate carrying the *bclC* gene of *B. cenocepacia* H111; Km^r	This study, A. Grunau
pJTR2	pBBR1MCS containing the *cepR2* gene of *B. cenocepacia* H111; Cm^r	This study
pKD4	kanamycin cassette template; Amp^r, Km^r	(Datsenko & Wanner, 2000)
pRN3	promoter probe vector, pSU11 derivative; Tp^r	This study
pRK2013	RK2 derivative, *mob*$^+$ *tra*$^+$ *ori* ColE1; Km^r	(Figurski & Helinski, 1979)
pSU11	promoter probe vector; Gm^r	S. Uehlinger,
P_{aidA1}-*lacZ*	pSU11/pRN3 containing a long H111 *aidA* promoter region; Gm^r/ Tp^r	S. Uehlinger
P_{araC}-*lacZ*	pSU11/pRN3 containing the putative H111 *bclA* promoter; Gm^r/ Tp^r	This study
P_{bclA}-*lacZ*	pSU11/pRN3 containing the putative *bclA* promoter region; Gm^r/ Tp^r	This study
$P_{cepIlong}$-*lacZ*	pRN3 containing H111 *cepI* promoter region (416 bp); Tp^r	This study
P_{cepR2}-*lacZ*	pRN3 containing the putative H111 *cepR2* promoter region; Tp^r	This study
P_{pchD}-*lacZ*	pSU11/pRN3 containing putative H111 *pchD* promoter region; Gm^r/ Tp^r	This study
P_{pchR}-*lacZ*	pSU11 containing the putative H111 *pchR* promoter region; Gm^r/ Tp^r	This study

Table 1. Bacterial strains and plasmids used in this study. Antibiotic resistances are: Ampicillin (Ap^r), Chloramphenicol (Cm^r), Gentamicin (Gm^r), Kanamycin (Km^r), Streptomycin (Sm^r) and Trimethoprim (Tp^r).

2.1.2 Media, chemicals and other material

Culture media used in this study are listed below, antibiotics and other supplements which were employed in this work are given in Table 2. Unless otherwise stated all media were autoclaved at 121°C for 20 min and supplements or other chemicals were subsequently added. Solid media contained routinely 1.5% (w/v) agar, soft agar contained 0.75% agar. *Pseudomonadae* and *Burkholderiae* were selected on PIA-agar (Becton Dickinson, Switzerland) which was prepared according to the instructions of the manufacturers. All media were ordered from Becton Dickinson (Switzerland), chemicals were purchased from Fluka (Buchs, Switzerland) or Sigma-Aldrich (Buchs, Switzerland). Enzymes and material used for molecular work were purchased from various companies including Invitrogen (Basel, Switzerland), MBI Fermentas (Glen Burnie, USA, New England Biolabs (Ipswich, USA),

Promega (Madison, USA), Roche (Rotkreuz, Switzerland) and Ambion (Austin, USA). The composition of buffers is described along with the experiments.

ABC/ ABG minimal medium (Clark & Maaløe, 1967)

For V = 1 liter autoclave component A and B separately, then combine 1 part of A with component B (9 parts) and add citrate (C) or glucose (G) as a carbon source.

Component A (10x)	
$(NH_4)_2SO_4$	0.15 M
Na_2HPO_4	0.42 M
KH_2PO_4	0.22 M
NaCl	0.51 M

Component C (final concentration)
10 mM sodium citrate or 10 mM D-glucose

Component B (for V = 1 liter)	
1 M $MgCl_2$ x 6 H_2O	2.0 ml
0.5 M $CaCl_2$ x 2 H_2O	0.2 ml
0.01 M $FeCl_3$ x 6 H_2O	0.3 ml
dH_20	ad 900 ml

FAB-medium (modified) (Heydorn *et al.*, 2000)

Components are essentially as in AB minimal medium with the following exception: the component B contains trace metal solution (1 ml/l of 10x solution, see below) instead of $FeCl_3$.

10x trace metal solution	
$CaSO_4$ x $2H_2O$	0.2 g/100 ml
FeSO4 x 7H2O	0.2 g/100 ml
$CuSO_4$ x $5H_2O$	0.02 g/100 ml
$ZnSO_4$ x $7H_2O$	0.02 g/100 ml
$MnSO_4$ x H2O	0.02 g/100 ml
$CoSO_4$ x $7H_2O$	0.01 g/100 ml
$NaMoO_4$ x H_2O	0.01 g/100 ml
H_3BO_3	0.005 g/100 ml

LB-medium (Luria Bertani-medium) (Andersen *et al*., 1998)

Caseinhydrolysate (Tryptone)	1% (w/v)
Yeast extract	0.5%
NaCl	0.4%
	pH 7.4

M9 medium (Johnson *et al.*, 2008)

5x M9 salts	
Na_2HPO_4 x 7 H_2O	64.0 g
KH_2PO_4	15.0 g
NaCl	2.5 g
NH_4Cl	5.0 g
dH_20	ad 1000 ml

Mineral Salt Medium (Lindhardt TJ, 1989)

$NaNO_3$	15.0 g
KCl	1.1 g
NaCl	1.1 g
$FeSO_4$ x 7 H_2O	0.00028 g
KH_2PO_4	3.4 g
K_2HPO_4	4.4 g
$MgSO_4$ x 7 H_2O	0.5 g
Yeast extract	0.5 g
dH_20	ad 1000 ml

SOC-medium (Sambrook, 1989)

Tryptone	2%
Yeast	0.5%
NaCl	10 mM
$MgCl_2$	10 mM
$MgS0_4$	10 mM
D-glucose	20 mM
	pH 7.0

Succinate medium (Meyer, 1978)

K_2HPO_4	6.0 g
KH_2PO_4	3.0 g
$(NH_4)_2SO_4$	1.0 g
$MgSO_4$ x $7H_2O$	0.2 g
Succinic acid	4.0 g
dH_20	ad 1000 ml; pH 7.0

Chromazurol-S (CAS) agar

See Material and Methods (2.4.4).

Swarming/ Swimming-agar

Components are ABC minimal medium with 0.2% (w/v) agar (swimming agar) or 0.4% (w/v) agar (swarming agar) supplemented with casamino acids [0.1%].

Milk-agar

LB-agar supplemented with 2% (w/v) skim milk powder in dH_2O (boiled for sterilization).

Substrate	Stock concentration	Final concentration	Solvent
Ampicillin	100 mg/ml	100 µg/ml	50% EtOH
Chloramphenicol	20 mg/ml	25 µg/ml	50% EtOH
Gentamicin	20 mg/ml	20 µg/ml	dH_20
Kanamycin	50 mg/ml	50 µg/ml	dH_20
Streptomycin	50 mg/ml	50 µg/ml	dH_20
Trimethroprim	100 mg/ml	100 µg/ml	DMSO
Casamino acids	20% (w/v)	0.1% (w/v)	dH_20
Cholesterol	10 mg/ml	5 µg/ml	100% EtOH
IPTG	100 mM	100 µM	dH_20
Paraformaldehyde	4% (w/v)	4% (w/v)	PBS, ad NaOH
Propidiume iodide	2 mM	0.5 µM	DMSO
Uracil	2 mg/ml	2 µg/ml	dH_20, heat
X-Galactose	40 mg/ml	40 µg/ml	DMF

Table 2. Antibiotics and other supplements used in this study (stored at -20°C).

2.1.3 Oligonucleotides

Oligonucleotide primers were ordered exclusively from MWG Biotech AG (Ebersberg, Germany) and are listed in Table 3. Unless otherwise noted, primers were designed based on the *B. cenocepacia* J2315 sequence (Holden *et al*., 2009).

Oligonucleotide	Sequence 5'-3'	Reference
araCP-F	GGCTCGAGCGTGCAGTATTGTCAGGTC	This study
araCP-R	GGAAGCTTGTCTTGAACGCTGGTCATGG	This study
cepR2-F	CGCAAGCTTGCTCGGATTCCGGTA	This study
cepR2-R	ACCAAGCTTGAGAGGCTCCGCACAGG	This study
cepR2pBBR-F	CGCAAGCTTCTT TTGAACAGGATACGC	This study
cepR2pBBR-R	TGCACTAGTCATAAGCGGGTCGAAAC	This study
cepR2P-F	GGAAGCTTGTCTTGAACGCTGGTCATGG	This study
cepR2P-R	GGCTCGAGCGTGCAGTATTGTCAGGT	This study
cepVpBBR-F	CGGATATCTTCCGCGCATGACTTCACC	This study
cepVpBBR-R	TAGGATCCGTTGTCACTGGAGCGTCG	This study
cepVpBBR-F_FS	CGGATATCTTCCGCGCATGACTCTCACCCTTGCT	This study
cepVpBBR-R_FS	TAGGATCCGTTGTCATGGAGCGTCGGAC	This study
cepVupGW	TACAAAAAAGCAGGCTACCGACAATCCGAATCGCG	A. Carlier
cepVDnGW	TACAAGAAAGCTGGGTACATCCGCACATGTCGCCGT	A. Carlier
cepVUpRkan	GAACTTCGAAGCAGCTCCAGCCTAACATGGTCGAGCACGAGTGC	A. Carlier
cepVDnFkan	CGGAATAGGAACTAAGGAGGATATTCATATGACATCGGCATGTTGCGCACG	A. Carlier
GW-attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCT	Invitrogen
GW-attB2	GGGGACCACTTTGTACAAGAAAGCTGGGT	Invitrogen
kanDnFwd	CGAAATGACCGACCAAGCGA	This study
kanUpRev	ACGTGTTCCGCTTCCTTTAGC	This study
lacZ-R	TGCTGCAAGGCGATTAAG	This study
lecB2-dn	GAATGGAAGGTTCCGCATCG	This study
lecB2-up	TGACGCACGTTCGACGA	This study
lecB3-F	TTCCATGGCACAACCCTT TACCCACG	This study
lecB3-R_his	AACTCGAGACCCAGCGGCCAGTTCAGG	This study
lecBP4	GGAAGCTTTCATGATGTCGGTCCTCGGT	This study
lecDn-GW	TACAAGAAAGCTGGGTTAGCGTGCGTCAACCCAGCG	This study
lecDn-kan	CGGAATAGGAACTAAGGAGGATATTTCATATG	This study
lecUp-GW	TACAAAAAAGCAGGCTCGCCGGTGAGGCCCTATTCATG	This study
lecUp-kan	GAACTTCGAAGCAGCTCCAGCCTAGCGCGGTTGGATGACGTTG	This study
M13-F	TGTAAAACGACGGCCAG	Standard
M13-R	CAGGAAACAGCTATGACC	Standard
PcepI-F	GGCTCGAGGGCCGCGATTCCTCTGACG	S. Uehlinger
PcepI-R	GGAAGCTTGCCGATAGCGCCCGAGATCC	S. Uehlinger
PcepVdnXho	GGCTCGAGATCGGCATGTTGCGCACG	This study
PlecB-dn	GGCTCGAGAGTCGTAGCGAGAAGAGGA	This study
PlecB-up	GGAAGCTTTTGAGAATCAGCCATGCGT	This study
pyo2231P-F	GGCTCGAGGAGGAAGGGGAGGATT	This study
pyo2231P-R	GAAAGCTTGGTGACGTCGTGACGAA	This study
pyo2232P-F	CACTCGAGGTGACGAAGCGAAGAA	This study
pyo2232P-R	GGAAGCTTGAGGAAGGGGAGGATT	This study

Table 3. Oligonucleotides used in this study. Restriction endonuclease sites are underlined. All primers were diluted to working concentrations of 20 µM.

2.2 Molecular biological methods

2.2.1 Cultivation of bacterial strains and growth conditions

Unless otherwise stated bacterial strains were incubated at 30°C or 37°C in liquid or solid media as listed previously. Liquid cultures were grown while shaking (225 rpm). *Pseudomonadae* and *Burkholderia* were selected on PIA-agar plates (2.1.2). For the selection of strains or plasmids antibiotics were added to final concentrations as listed (Table 2, 2.1.2). Bacterial strains were stored in 15% glycerol at -80°C.

2.2.2 Manipulation of DNA

2.2.2.1 DNA preparation and characterization

Analytical amounts of plasmid DNA were isolated as described (Birnboim, 1983), and preparative amounts were purified with Qiagen columns following the instructions of the manufacturers (Qiagen, Hilden, Germany). Chromosomal DNA (total DNA) of *B. cenocepacia* was isolated by sarcosylpronase lysis as previously described (Better *et al.*, 1983). Briefly, 2 ml of a bacterial overnight culture were precipitated and the pellet was resuspended in 2 ml of TE-buffer (50 mM Tris, 20 mM EDTA, pH 8). To this bacterial suspension, 2.5 ml of 2% sarcosyl (Sigma, Buchs, Switzerland) were added and the mix was homogenized by inverting the tube. After the addition of 0.5 ml of pronase (5mg/ml, Sigma, Buchs, Switzerland) and tube invertion the cells were incubated at 37°C until the solution has cleared (10 - 30 min). The sample was vortexed (2 min) and the cell lysate was extracted once with phenol/chloroform/*iso*-amyl alcohol (25:24:1) and then once with chloroform (Sigma, Buchs, Switzerland). After centrifugation (5000 rpm, 5 min), the liquid phase was transferred to a new collection tube and DNA was precipitated by adding NaCl (stock solution: 5 M, final concentration: 0.2 M) and an equal volume of isopropanol. After inverting the tube the DNA was pelleted by centrifugation (max. speed, 15 min), shortly washed with 70% ethanol (pipette tip), air-dried and resuspended in 500 µl TE-buffer.

DNA characterization was achieved employing standard techniques (Sambrook *et al.*, 1989) including agarose gel electrophoresis, polymerase chain reactions (PCR) and restriction analyses with endonucleases. Positive transformants were initially detected by direct PCR (colony-PCR) with DNA deriving from bacterial liquid cultures (1 µl) or bacterial cultures grown on agar plates. DNA was added to 4 µl of lysis mixture (50 mM KCl, 0.1% Tween-20, 10 mM Tris-HCl, pH 8.3) and PCR reactions were performed routinely. DNA was extracted from agarose gels using a Qiaquick gel extraction kit (Qiagen, Hilden, Germany) and further

concentrated if nessecary by standard ethanol precipitation (Sambrook *et al*., 1989). Southern blot hybridisation was employed for the characterization of mutant H111-R2 as described below.

2.2.2.2 Southern blot hybridization

The mutation in *cepR2* (mutant H111-R2 and mutant H111-R/R2) was verified by Southern blot analyses employing standard techniques (Southern, 1975). Chromosomal DNA of the H111 wild type and of mutant H111-R2 or H111-R/R2 was digested with restriction endonucleases, reported by agarose electrophoreses and then transferred to a nylon membrane. The membrane was then hybridized to a labelled *cepR2*-RNA-polynucleotide probe. The probe was enzyme-linked and could therefore be visualized with a colorimetric reaction. The DNA probe was constructed as followed: a DNA fragment comprising *cepR2* encoding sequences (primers cepR2-R and cepR2-F, 2.1.3) was labelled with dioxygenin (DIG) using the Random Prime DNA Labeling Kit (Roche, Mannheim, Germany) following the instructions of the manufacturers. Approximately 5 to 10 µg of chromosomal DNA of the H111 wild type and mutant H111-R2 or mutant H111-R/R2 were digested with restriction enzyme *Bam*HI (37°C, include RNase A, overnight). *Bam*HI cleavage sites are among others present in the multiple cloning site of plasmid pEX18Gm, which was employed for the construction of the *cepR2* insertion mutants (2.2.4.1), and in the up- and downstream DNA regions of the *cepR2* gene (see Fig. 5, J. Toller, Master thesis, 2008). The DNA was then separated by electrophoresis (1% agarose gel), and the DIG labelled molecular marker II (Roche) was moreover employed as a control in the gel. After destaining to remove ethidium bromide the gel was washed in solution A (15 min, 0.25 M HCl), solution B (30 min, 0.5 M NaOH, 1.5 M NaCl) and solution C (40 min, 1 M Tris base, 1.5 M NaCl, pH 7.5), then blotted (pressure 80 mm Hg, 1 h) to a HybondTM-N^+ nylon membrane (Amersham, Cleveland, USA) in a Stratagene blotting chamber (Heidelberg, Germany). The DNA was then cross-linked to the dry membrane under UV light (3 min). Prior the addition of the polynucleotide probe, the membrane was pre-hybridized in DIG Easy Hyb buffer (Roche) at 52.8 °C for 4h. The hybridization temperature was thereby calculated according to the instructions of the manufacturers (see J. Toller, Master thesis, 2008). The polynucleotide probe was first denaturated (100°C, 10 min), cooled on ice, and added to the pre-hybridization buffer. After hybridization (overnight), the membrane was washed (2 x 5 min, RT) in 25 ml of wash solution 1 (2x SSC, 0.1% SDS [20x SSC: 3 M NaCl, 0.3 M sodium citrate, pH 7.0]), then (2 x 15 min, 68°C) in wash solution 2 (0.5x SSC, 0.1% SDS). After further incubation (5 min) in

maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5), the membrane was saturated (30 min) in blocking solution (10x blocking solution: 1:10 diluted maleic acid buffer) and finally incubated (30 min) in the antibody solution (150 mU/ml Anti-DIG AP conjugate in 1x blocking solution, Roche). The wash step in maleic acid buffer was repeated (2 x 15 min) followed by the equilibration of the membrane in detection buffer (0.1 M Tris base, 0.1 M NaCl, pH 9.5). For the colorimetric reaction, the membrane was transferred to a freshly prepared NBT/BCIP solution (200 µl NBT/BCIP (Roche) in 10 ml detection buffer) following incubation of the membrane for 1-3 h in the dark, until colored bands became visible.

2.2.2.3 Precipitation of DNA and RNA and quality analysis by gel electrophoresis

DNA was precipitated with ethanol by standard techniques (Sambrook *et al*., 1989). Briefly cations of the DNA solution were adjusted by adding sodium acetate (stock solution 3 M, pH 5.2) to a final concetration of 0.3 M. After adding two volumes of ice-cold ethanol (100%) the solution was mixed and stored on ice to allow precipitation (30 min). The DNA was pelleted by centrifugation (max. speed, 10 min, 4°C), the precipitate washed in 70% ethanol and the tube centrifuged (max. speed, 2 min, 4°C). The washing step was repeated once and the open tube stored at RT for the evaporation of remaining ethanol. The DNA pellet was resolved in TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8).

RNA was precipitated with lithium-chloride as follows: concentration of RNA samples were adjusted with 8 M LiCl to obtain a concentration of 2.5 M (use RNAse-free water, final volume of 1 ml). After incubuation of samples (30 min, -20°C), tubes were centrifuged (max. speed, 5 min, RT), and pellets were stored at RT to air-dry, then resuspended in ddH_2O (20 µl, RNase-free) and stored at -80°C. RNA quality (for further usage in the transcriptome analysis) was analysed by agarose gel electrophoresis as follows: 0.3 µl of the concentrated RNA samples (200 ng of RNA is good visilbe on a gel, mixed with 5 µl of ddH_2O and 3 µl of loading dye) were separated on a 0.5% agarose gel employing TAE-buffer (40 mM Tris-Acetat, 1 mM EDTA, pH 7.5). The gel was analysed for clear 16S and 23S rRNA bands (5S rRNA band is often difficult to see) and for smear (indicates DNA contamination and/or RNA degradation).

For inactivation of RNases all buffers were treated with DEPC (diethyl pyrocarbonate, final concentration: 0.1%). Here, DEPC was added to solutions and buffers which were incubated at 37°C (overnight), followed by the break-down of DEPC through autoclaving. In order to avoid enzyme degradation of RNA all equipment and the working place were cleaned with ethanol (100%), pipettes and plastic wear were moreover rinsed with 0.1 N NaOH.

2.2.2.4 Recombinant DNA techniques

Digestion with enzymes, agarose gel electrophoreses, purification of DNA fragments, dephosporylation of plasmid DNA, ligation with T4 DNA ligase, end filling with Klenow fragment of DNA polymerase were performed as described (Sambrook, 1989). Transformation of plasmid DNA in *E. coli* was performed by electroporation with a BioRad Gene pulser (BioRad, Hercules, USA) at 200 Ω, 25 µF and 2.5 kV. Glycerol-treated electrocompetent cells were prepared as described (Sambrook *et al*., 1989). Cells were resuspended in SOC-medium (see 2.1.2) following the transformation and incubated at 37°C (1h) prior the selection on agar plates containing appropriate antibiotics.

Tri-parental matings from *E. coli* to *B. cenocepacia* were performed with helper strains *E. coli* MM294 (pRK2013) and *E. coli* HB101 (pRK600) (2.1.1) according to a previously described procedure (De Lorenzo & Timmis, 1994). Briefly, overnight cultures of the donor strain, the helper strain, and the *Burkholderia* recipient strain were harvested and washed in LB-medium to remove residual antibiotics. Culture samples of the donor and the helper strain (100 µl each) were mixed and incubated for 10 min at RT. A volume of 200 µl of the recipient strain was added and the mixture was plated in 50 µl spots on prewarmed LB-agar plates. After incubation at 37°C (at least 8h or overnight) bacteria were resuspended in volumes of 0.9% sodium chloride, transferred to selective PIA agar plates and further incubated at 37°C.

2.2.3 DNA sequence determination and database analysis

Sequencing reactions were performed employing the ABI BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Briefly, DNA (ca. 100-180 ng) was mixed with 0.8 µl BigDye, 0.5 µM primer, 1x sequencing buffer and 5% DMSO in a total volume of 10 µl. Sequencing reactions were routinely performed as followed: 94°C for 2 min, 96°C for 10 s, 50°C for 5 s, 60°C for 3 min; applying 60 cycles. Subsequent purification and electrophoresis steps were performed using the ABI 3730 DNA analyzer (Applied Biosystems, Institute sequencing service) and analysed with the software Chromas 2.

The following databases and softwares were used for the analyses of DNA or protein sequences/ structure: KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/), the Burkholderia genome database, http://www.burkholderia.com/), The Welcome Trust Sanger Institute, http://www.sanger.ac.uk/Projects/B_cenocepacia/, NCBI (http://www.ncbi.nlm.nih.gov/), EMBL (http://www.embl.de/), Softberry, http://linux1.softberry.com/berry.phtml), DOE Joint Genome Institute, http://www.jgi.doe.gov/), CLC DNA workbench, iTASSER

(http://zhanglab.ccmb.med.umich.edu/I-TASSER/) and Phyre (http://www.sbg.bio.ic.ac.uk/~phyre/).

2.2.4 Allelic replacement and mutagenesis in *B. cenocepacia* H111

2.2.4.1 Generation of mutant H111-R2 and H111-R/R2

A H111 *cepR2* mutant (designated as H111-R2) was constructed as follows: a 384 bp internal fragment of *cepR2* was amplified with primers cepR2-R and cepR2-F (2.1.3), digested with *Hind*III, ligated into the gene replacement vector pEX18Gm (2.1.1), and transformed into *E. coli* XL1-Blue. The final construct was transferred to H111 by triparental mating according to De Lorenzo *et al.* (1994) and allelic exchange of mutant strains was later confirmed by PCR and southern blot hybridization (see Results: 3.2 and Master thesis of J. Toller, 2008). The H111 *cepR cepR2* double mutant, designated H111-R/R2, was constructed in an analogous manner using the H111 *cepR* mutant (H111-R) as the recipient strain (see 3.2).

2.2.4.2 Generation of deletion mutant H111-*bclACB* and H111-rsaM

A lectin deficient H111 mutant, referred to as H111-*bclABC*, was generated by allelic replacement of the lectin-encoding operon using a modified protocol of the Gateway cloning technology (Carlier *et al*., 2009). Briefly, flanking regions of *bclA* and *bclB* were amplified by PCR using the oligonucleotides lecDn-GW and lecDn-kan and lecUp-kan and lecUp-GW (2.1.3 and 3.3). A kanamycin cassette derived from plasmid pKD4 (2.1.1) was then inserted between the flanking regions by means of an overlap PCR reaction. The resulting PCR products were cloned into the Gateway Entry vector pDONR221 (2.1.1) using BP Enzyme II Mix (Invitrogen, Carlbach, USA) and then transferred into the suicide vector pAUC40 (2.1.1) using the LR Enzyme II Mix (Invitrogen, Carlbach, USA) following the instructions supplied by the manufacturer. The resulting plasmid (pAUC40-bcl, see 3.3) was conjugated into *B. cenocepacia* by triparental mating (2.2.2.4), and transconjugants were selected for kanamycin-resistence and streptomycin-sensitivity. Allelic replacements were verified by PCR using oligonucleotides lecB2-up and lecB2-dn and primers KanUpRev and KanDnFwd (2.1.3 and 3.3) and by Western immunoblotting with BclC antibodies (2.3.4 and 3.3). The H111 *rsaM* (BCAM1869) deletion mutant H111-rsaM was contructed by A. Carlier using the Gateway cloning technology analogous as described above. Primers used for the mutagenesis are: cepVupGW, cepVDnGW, cepVUpkan and cepVDnFkan (Table 3, 2.1.3).

2.2.5 Construction of plasmid pJTR2, pBBR*rsaM* and pBBR*rsaM*_FS

For complementation analyses we constructed the *cepR2* expressing plasmid pJTR2, plasmid pBBR*rsaM*, expressing the *rsaM* gene, and plasmid pBBR*rsaM*_FS, which encodes a framshiftet ORF of *rsaM*. The *cepR2* ORF was amplified from genomic DNA of H111 by PCR employing primers cepR2pBBR-F and cepR2pBBR-R (2.1.3). Subsequently the DNA was first subcloned into plasmid pCR2.1Topo (Invitrogen) and then inserted into plasmid pBBR1MCS (2.1.1) using the *Eco*RV and *Bam*HI restriction sites to create pJTR2 (for plasmid map see Master thesis J. Toller, 2008).

For the construction of plasmid pBBR*rsaM* a 572 bp DNA fragment comprising the coding-sequences of the predicted *rsaM* gene was amplified by PCR with primers cepVpBBR-F and cepVpBBR-R (2.1.3). A frameshifted *rsaM* ORF (followed by a translational STOP codon) was generated employing primers cepVpBBR-F_FS and cepVpBBR-R_FS (2.1.3). Amplicons of DNA sequences were generated by PCR and inserted into plasmid pBBR1MCS as described above (if necessary the DNA was subcloned into plasmid pCR2.1Topo, Invitrogen).

2.2.6 Generation of plasmid pJBA89luxR$^-$ by partial digestion

Plasmid pJBA89*luxR* $^-$ was constructed by partial digestion of pJBA89 with restriction enzymes *Eco*RI and *Hind*III, in which a 703 bp DNA fragment containing *luxR* (*Hind*III-digestion side 55 bp downstream of the start codon) was removed from plasmid pJBA89. Protruding 3'-termini were filled in with Klenow fragment and blunt ends were reunited employing T4-ligase.

2.2.7 Generation of plasmid pET-HisBclB

The BclB (LecB3)-encoding sequence was amplified by PCR employing primers lecB3-F and lecB3-R_his (2.1.3). The amplicon was inserted into pCR2.1 Topo (Invitrogen), cut-out off the vector and cloned into plasmid pET28a (Novagen, Madison, USA) to generate a translational fusion of the histidine tag and *bclB*. For protein overexpression, see 2.3.3. Cloning was done by Alexander Grunau.

2.2.8 Transcriptional fusions to *lacZ*

Transcriptional fusions were constructed encoding upstream regions of putative promoters of the following genes: *araC* (BCAM0189), *bclA*, *cepI*, *cepR2*, *pchD* and *pchR*. Promoter regions were amplified by PCR using oligonucleotides araCP-F, araCP-R; PlecB-up, PlecB-

dn; PcepI-F, PcepI-R; PcepI-F, CepVdn-Xho; cepR2P-F, cepR2P-R; pyo2231P-F, pyo2231P-R and pyo2232P-F, pyo2232P-R (Table 3, 2.1.3). The PCR fragments were cloned as *Xho*I and *Hind*III fragments into the respective sites of the promoter-probe vector pSU11 or pRN3 (see 2.1.1) generating plasmids P_{araC}-*lacZ*, P_{bclA}-*lacZ*, P_{cepI}-*lacZ*, $P_{cepIlong}$-*lacZ*, P_{pchD}-*lacZ* and P_{pchR}-*lacZ* (Table 1, 2.1.1). Promoter fusion P_{aidA1}-*lacZ* comprises a long fragment of the *aidA* promoter (*aidA*1, see S. Uehlinger, Dissertation, 2009). The transcriptional fusions were analysed by PCR employing the primers listed above and primer lacZ-R (2.1.3). Plasmids were transferred to *B. cenocepacia* by means of triparental mating (2.2.2.4).

2.2.9 RNA extraction and transcriptome analysis

For the extraction of RNA, overnight cultures of planktonic cells were inoculated in 10 ml LB-medium to an OD_{600} of 0.02. The following strains were employed for the microarray profiling: the H111 wild type, the *cepR* mutant H111-R, and the plasmid pBAH27 (2.1.1) harbouring complemented strain H111-R (*cepR*$^+$). Cells were harvested at stationary phase growth at an OD_{600} of 2.5, because the expression of *aidA* (a stringently QS-regulated virulence factor, see 3.1) as well as production of signal molecules was found to be maximal at this density. RNA was extracted and purified as recommended by the RiboPure^{TM}-Bacteria Kit (Ambion) with the following modifications: the lysate-ethanol mixture from two tubes of each sample was transferred to one filter cartridge to improve RNA yield. To remove trace amounts of genomic DNA extracts were treated with DNase I for 60 minutes at 37°C. RNA concentration and integrity was monitored applying NanoDrop and agarose gel electrophoresis.

Two-color microarray experiments were performed at the School of Biosciences at Cardiff University using Agilent 4-pack BCC gene chips, which were designed on the basis of the sequenced *Burkholderia cenocepacia* J2315 genome (Holden *et al*., 2009). The gene chip comprised 10264 gene probes with 8741 sequences of *B. cenocepacia* strain J2315, 1070 gene sequences belonging to *Burkholderia cenocepacia* strain AU1054 and 387 gene sequences of *Burkholderia cenocepacia* HI2424. In total, 3 biological replicates of each of the analysed strains, the H111 wild type (control), mutant H111-R and H111-R (*cepR*$^+$) were prepared. Total RNA (10-20 µg) was used for synthesis of cDNA. Labelling of first strand cDNA was performed according to the CyScribe Post-Labelling Kit Protokol (GE Healthcare) as briefly followed: random nonamers, anchored oligonucleotides and control spikes (Two-Color Microarray-Based Gene Expression Analysis, Agilent) were added to the RNA samples and allowed to anneal to the mRNA (70°C for 5 min, RT for 10 min). In the second step cDNA

was synthesized using CyScript reverse transcriptase incorporating aminoallyl-dUTP according to the manufactor´s instruction (GE Healthcare Amersham CyScribe Post-Labelling Kit). RNA was removed by alkaline treatment and subsequent neutralization. After purification of cDNA (CyScribe GFX Purification Kit, GE Healthcare), the cDNA was coupled with CyDye NHS ester. The CyDye labelled cDNA was again purified (CyScribe Post-Labelling Kit, GE Healthcare). Hybridizing the DNA to the microarray (Two-Color Microarray-Based Gene Expression Analysis Kit, Agilent, and GE hybridization buffer) control-DNA (Cystic fibrosis foundation therapeutics (CFFT) at the University of North Carolina) was again incorporated. Microarray washing was performed according to the instructions of the manufacturer (Agilent).

Transcriptome data processing and interpretation

Transcriptome analyses were performed in colaboration with the CFFT (cystic fibrosis foundation therapeutics) at the University of North Carolina. Signal intensities were first detected and analysed towards quality with Agilent Feature extraction software (v.9.5). The dataset was then incorporated into GeneSpring (v.7.3.1) and processed using the "Affimetrix FE" data normalization procedure recommended for Agilent arrays, i.e., first data transformation to set measurements of below 0.01 to 0.01 followed by a per-chip normalization to the 50th percentile and a per-spot normalization to median. Incorrect probes as well as genes, which were not present in at least 3 arrays, were further excluded from the data set. The remaining probes were filtered using a fold change value of ≥3. Statistical analysis of the remaining genes was carried out using ANOVA along with the "Benjamini and Hochberg False Discovery Rate (BH_FDR) multiple testing correction". Differentially expressed genes that hybridized to array probes belonging to *B. cenocepacia* strain AU1054 including CDSs for genes (6), tRNA genes (19 CDSs), as well as intergenetic regions (21 CDSs), were excluded in this study.

2.3 Protein biochemical methods

2.3.1 Extraction of proteins and determination of protein amount

The extraction of extracellular and whole cell protein fractions which were analysed by iTRAQ is described in 2.3.6. For the Western immunoblot analyses of BclB (2.3.4) we employed extracellular and whole cell protein samples of planktonic cells, which were prepared as followed: *B. cenocepacia* H111 wt and mutants were grown while shaking in LB-medium (V = 300 ml, 37°C, inoculation from over night culture to an OD_{600} of 0.1). 50 ml

culture samples were collected at different cellular densities as determined by absorbance at 600 nm over a period of 24h (see 3.3). After centrifugation (5000 rpm, 30 min, 4°C), supernatants were transferred to a schott bottle (for the precipitation of extracellur proteins) and cell pellets were washed in 50 ml PBS, then resuspended in 20 ml of 50 mM Tris-HCl, pH 7.5, 1% SDS. In order to concentrate and adjust cell densities of the probes, samples were centrifugated (5000 rpm, 30 min, 4°C) and cell pellets were resuspended in 50 mM Tris-HCl, pH 7.5, 1% SDS with final volumes ranging from 400 to 6000 µl. (The cell pellet deriving from a cell cultures of 20 ml with a measured OD_{600} of 1.0 was resuspended in 400 µl of buffer, other samples were resolved proportional). The protein concentration was quantified according to Bradford (Bradford, 1976) employing the Pierce Protein Assay (Pierce, Rockford, USA). Protein amounts of the samples determined by Bradford were approximately 2 mg/ml.

Extracellular proteins (see above) were extracted with TCA (15%, w/v) and samples were incubated overnight for protein precipitation under agitation at 4°C. After pelleting (5000 rpm, 90 min, 4°C) proteins were once washed with 70% ethanol then washed with ice-cold acetone and again centrifuged (8500 rpm, 20 min, 4°C). Pellets were air-dried and solved in a final volume of 400 µl 50 mM Tris-HCl, pH 7.5, 1% SDS. Protein amounts determined by Bradford were approximately 2 mg/ml). In all cases, equal amounts of protein (30 µl of protein samples) were mixed with spratt buffer, partially sonicated, and loaded on a 15% SDS gel for gel electrophoreses as described below.

2.3.2 SDS-gel electrophoresis and protein visualization

For analytical analyses proteins were routinely separated according to their molecular weight through a discontinued sodium dodecyl sulfate gel electrophoresis (SDS–PAGE) (Laemmli, 1970). The composition of a 15% SDS-gel consisting of the two denaturing gels, the stacking-gel (5% (w/v) acrylamide) and the separating-gel (15% (w/v) acrylamide) is listed in Table 4.

Gel (% Acrylamide)	**Acrylamide[a)]**	**Buffer**	**dH_2O**	**APS[d)]/ Temed[e)]**
Stacking-gel (5%)	3.34 ml	5 ml buffer [b)]	11.6 ml	150 µl/ 15 µl
Separating-gel (15%)	20 ml	10 ml buffer [c)]	10 ml	250 µl/ 25 µl

Table 4. Composition scheme for the preparation of three 15% SDS-gels (V = 20 ml each).
[a)]: 30% Acrylamid, 1.5% N,N-Methylenbisacrylamid in dH_2O
[b)]: 0.5 M Tris-HCl, pH 6.8
[c)]: 1.5 M Tris-HCl, pH 8.8
[d)]: TEMED = N,N,N',N' – Tetramethylendiamin
[e)]: APS =Ammonium peroxodisulfate

Prior the probe application on the gel, samples were combined with spratt buffer (1/3 of the volume; 50 mM Tris-HCl, pH 6.8, 40% (w/v) glycerol, 6% (w/v) SDS, 15% (w/v) glycerol, 5% (v/v) ß-mercaptoethanol and some bromophenol blue cristals), and boiled for 10 min at 100°C. The samples were centrifuged and those containing whole cells proteins were furthermore exposed to sonication (40% amplitude, 30 s). Electrophoresis was performed employing standard electrophoresis buffer (20 mM Tris, 192 mM Glycin, 0.1% SDS (w/v) and applying an initial current of 15 mA (45 min) followed by a current at 35 mA (1 h). Protein standards were included as a marker (precision plus protein standards all-blue, BioRad). If the gel was not furter employed for Western immunoblotting, proteins were subsequently visualized by staining with the dye Serva Blue G (Serva, Heidelberg, Germany) according to the instructions of the manufacturers.

2.3.3 Overproduction and purification of lectin BclB (BCAM0184)

For protein overexpression plasmid pET-HisBclB (2.2.7) was transformed into *E. coli* BL21 (DE3) and gene expression was induced by the addition of 1 mM isopropyl-l-thio-β-D-galactopyranoside. BclB expression was analysed by SDS-PAGE as described (2.3.2). Purification of BclB was performed by affinity-chromatography on a Ni-NTA-HisTrap FF column (GE Healthcare) followed by gel filtration on a Superose 12 10/300 GL column (GE Healthcare) using an ÄKTA Purifier 10-UPC950 FPLC system, GE Healthcare).

2.3.4 Generation of BclB antibodies and Western immunoblot analysis

Polyclonal antibodies directed against BclB were generated using the purified protein (Coring System Diagnostix GmbH, Germany). The immunoblotting was conducted employing fractions of whole cell proteins or extracellular proteins. All steps were performed at RT. Protein samples (2 mg/ml protein, see 2.3.1) were loaded on a 15% SDS gel (2.3.2) and separated by electrophoresis. Protein blotting was prepared by transferring nine sheets of Whatman paper (3MM, Whatman, Maidstone, UK) soaked in transfer buffer (48 mM Tris, 38 mM glycine, 0.04% SDS (w/v), 20% methanol (v/v), pH 8.3) to the anode of a semi-dry blotting apparatus (NovaBlot 2117 250, GE Healthcare, UK). A polyvinylidene difluoride (PVDF) membrane (Amersham Hybond^{TM}-P, GE-Healthcare, cut to the size of the gel) was quickly equilibrated in methanol and transfer buffer and placed onto the paper stack avoiding the formation of bubbles. After a 20 min incubation period in transfer buffer, the gel was subsequently transferred to the membrane, covered with nine additional sheets of Whatman

paper (soaked in transfer buffer) and sealed by the cathode of the apparatus. Protein transfer was conducted at 0.8 mA/cm^2 gel for 1 h.

For the subsequent detection of BclB the membrane was washed twice (each 10 min) in TBS buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl), then incubated for 1 h in blocking solution (TBS buffer containing 3 % (w/v) bovine serum albumine). Following two washing steps (10 min each) in TBS-T buffer (20 mM Tris-HCl, pH 7.5, 500 mM NaCl; 0.05% Tween-20 (v/v), 0.2% Triton-X100 (v/v)) and one incubation period in TBS buffer, the membrane was transferred to the binding solution (one volume blocking solution, one volume TBS buffer), which was supplemented with the BclB-antibody (1: 5000). Antibody binding was allowed for 1h and the membane was again washed prior incubation with the second antibody (twice in TBS-T buffer, once in TBS buffer, 10 min each). Following 1 h of incubation in binding buffer containing alkaline-phosphate conjugated anti-rabbit immunoglobulin G (1:7000, Sigma, Deisenhofen, Germany), the membrane was exposed to four final washing steps in TBS-T buffer and subsequently equilibrated in reaction buffer (100 mM Tris-HCl, pH 9.5; 100 mM NaCl, 5 mM $MgCl_2$). Enzyme activity was detected employing NBT/BCIP dye (200 µl in 10 ml reaction buffer, Roche, Penzberg, Germany) according to the instructions of the manufacturers.

2.3.5 Detection of BclB by immunofluorescence microscopy

To determine the subcellular localization of the BclB lectin in *B. cenocepacia* H111, polyclonal antibodies directed against the BclB protein (2.3.4) were labelled with the dye Alexa Fluor 594 (Invitrogen) according to the instructions of the manufacturer (the labelling was done by Alexander Grunau). The calculated concentration of labelled protein measured by absorbance at 280 nm and 590 nm (instructions of the manufacturers) was 0.2 mg/ml in PBS. For the assay we employed cells of bacterial cultures (100 ml volumes) which were grown at 37°C with agitation to an OD_{600} of 2.5. Bacterial cells (pelleted from 500 µl volumes) were fixed by incubation in 4% para-formaldehyde in PBS (10 min, RT), followed by a washing step with PBS. To permealize cell membranes and enable antibody binding to intracellular proteins samples aliquots were moreover incubated in PBS containing 0.2% Triton X-100 (10 min, RT) and subsequently washed with PBS. To reduce non-specific binding of the antibodies the cells were incubated in 1% bovine serum albumin in PBS (40 min, RT). All cellular preparations were washed twice with PBS and resuspended in a final volume of 50 µl. Cell labelling was achieved by adding the Alexa 594-conjugated BclB antibodies (4:5 diluted in cellular sample preparations), followed by incubation in the dark for

2h at RT. Labelling reactions were further kept at 4°C overnight, then washed with PBS (three times) and analysed by fluorescence microscopy (Center of Microscopy, University of Zurich).

2.3.6 Protein extraction and analysis by iTRAQ (isobaric tag for relative and absolute quantitation)

For comparative proteome analyses 500 ml LB-medium in 3 l Erlenmeyerflasks were inoculated with overnight cultures of the H111 wild type, the *cepR* mutant H111-R, and the *cepI* mutant H111-I (2.1.1) to an OD_{600} of 0.05 and incubated at 37°C under vigorous shaking (225 rpm). One out of two H111-I cultures was supplemented with 1 μM C8-HSL. To optimize the proteome-transcriptome comparison, cells were harvested at an OD_{600} of 3.0, where the abundance of AidA has been observed highest in the wild type strain. After centrifugation (5000 rpm, 15 min) supernatants were separated from bacterial cell pellets and sterile filtered.

Extracellular (EC) proteins were precipitated from sterile filtered culture supernatants with 15% trichloroacetic acid (TCA). Protein pellets were washed with acetone, dried at RT and resuspended in 50 mM Tris-HCl, pH 7.5. Proteins were further purified by phenol extraction as described elsewhere (Riedel *et al.*, 2003). To extract whole cell (WC) proteins bacteria cell pellets were resuspended in 4 ml of 0.5 M triethylammonium bicarbonate (TEAB) buffer and sonicated four times for 1 min on ice. After pelletting the cell debris (20.000 rpm, 30 min), proteins were precipitated from the supernatant with 6 volumes of acetone at -20°C overnight. Following centrifugation (20.000 rpm, 20 min) pellets were again resolved in 0.5 M TEAB buffer and stored at -80°C. The protein concentration was quantified according to Bradford (2.3.1). Subsequently, 100 μg of each protein sample were dried and resolved in 20 μl 0.05% SDS as stated in the iTRAQ Reagents Protocol (Applied Biosystems, Foster City, USA). After reduction of proteins and alkylation of cysteine residues, peptides were digested with trypsin and labelled with iTRAQ reagents. Four independent protein analyses were performed, in which the peptides were tagged with the different iTRAQ labels. In analysis I (WC proteins) and analysis II (EC proteins) we used tag 117 for H111 wild type peptides, tag 116 for H111-R peptides, tag 115 for H111-I peptides, and tag 114 for AHL-complemented H111-I peptides. In analysis III (biological replicate WC proteins) and analysis IV (biological replicate EC proteins) we used tag 114 for H111 WT peptides, tag 115 H111-R peptides, tag 116 for H111-I peptides, and tag 117 for complemented H111-I peptides. Prior to LC-MS/MS the tagged peptides from each analysis were combined.

For analytical protein analyses samples were separated by sodium dodecyl sulfate gel electrophoresis (SDS–PAGE) and bands were visualized by Coomassie blue staining (Serva Blue G; Serva, Heidelberg, Germany, 2.3.2).

Strong cation exchange chromatography (SCX)

The iTRAQ samples were separated into 27 fractions on a cation-exchange column (2.1 mm x 200 mm SCX-column, PolySULPHOETHYL A, 5 µm, 300-Å, PolyLC, Columbia, USA) using the gradient solutions mobile phase A (10 mM KH_2PO_4, 25% acetonitrile, pH 3) and phase B (10 mM KH_2PO_4, 25% acetonitrile, and 35 mM KCl, pH 3). Peptides were eluted at a flow rate of 0.3 ml/min over the following gradient: 10 min 100% mobile phase A, 40 min 0-50% mobile phase B, 10 min 100% mobile phase B. Fractions were pooled to four master-fractions according to the SCX spectrum and purified using a C-18 column (Sep-Pak cartridge, Waters Corporation, USA).

Nano-LC separation and MALDI target spotting of tryptic peptides

Samples were further analysed by nano-liquid chromatography coupled to matrix-assisted laser desorption/ionization time-of flight/time-of-flight tandem mass spectrometry (nano-LC-MALDI-TOF/TOF MS). Peptide separation was performed on an Ultimate chromatography system (Dionex - LC Packings, Sunnyvale, CA) equipped with a Probot MALDI spotting device. 5 µl of the samples were injected by using a Famos autosampler (Dionex - LC Packings) and loaded directly onto a 75 µm x 150 mm reversed-phase column (PepMap 100, 3 mm; Dionex - LC Packings). Peptides were eluted at a flow rate of 300 nl/min by using the following gradient: 0-10 min, 0% solvent B; 10-105 min, 0-50% solvent B; and 105-115 min, 50-100% solvent B. Solvent A contained 0.1% TFA in 95:5 water/acetonitrile, and solvent B contained 0.1% TFA in 20:80 water/acetonitrile. For MALDI analysis, the column effluent was directly mixed with MALDI matrix (3 mg/ml a-cyano-4-hydroxycinnamic acid in 70 % acetonitrile/0.1 % TFA) at a flow rate of 1.1 µl/min via a µ-Tee fitting. Fractions were automatically deposited every 10 s onto a MALDI target plate (Applied Biosystems, Toronto, Canada) using a Probot micro fraction collector. A total of 416 spots were collected from each HPLC run.

MALDI-TOF/TOF mass spectrometry

MALDI plates were analysed on a 4800 MALDI TOF/TOF system (Applied Biosystems, Toronto, Canada) equipped with a Nd:YAG laser operating at 200 Hz. All mass spectra were

recorded in positive reflector mode and generated by accumulating data from 800 laser shots. First, MS spectra were recorded from peptide standards on each of the six calibration spots, and the default calibration parameters were updated. Second, MS spectra were recorded for all sample spots on the MALDI target plate (416 spots per sample, 4 samples per plate). The MS spectra were recalibrated internally based on the ion signal of neurotensin peptide (Sigma, Buchs, Switzerland). Spectral peaks that met the threshold criteria and were not on the exclusion list were included in the acquisition list for the MS/MS spectra. The following threshold criteria and settings were used: Mass range: 800 to 4000 Da; minimum signal-to-noise (S/N) for MS/MS acquisition: 100; maximum number of peaks/spot: 8. Peptide CID was performed at a collision energy of 1 kV and a collision gas pressure of approximately 2.5 x 10-6 Torr. During MS/MS data acquisition, a method with a stop condition was used. In this method, a minimum of 1000 shots (20 sub-spectra accumulated from 50 laser shots each) and a maximum of 2000 shots (40 sub-spectra) were allowed for each spectrum. The accumulation of additional laser shots was halted whenever at least 6 ion signals with a S/N of at least 60 were present in the accumulated MS/MS spectrum, in the region above m/z 200.

Protein identification and relative quantification

GPS (Global Proteomics Server) Explorer Software (Applied Biosystems, Foster City, USA) was used for submitting data acquired with the MALDI-TOF/TOF mass spectrometer for database searching. The MS and MS/MS data were searched using Mascot version 2.1.0 (Matrix Science, London, UK) as the search engine (Perkins *et al.*, 1999). The following search settings were used: maximum missed cleavages: 1; maximum number of signals per spectrum: 55; peptide mass tolerance: 35 ppm MS/MS tolerance: 0.2 or 0.25 Da. ITRAQ labelling of lysine and of the N-terminal amino group of peptides and methyl methanthiosulfonate (MMTS) derivatization of cysteine were specified as fixed modifications. All searches were performed against two databases (p142_burkhold, 148894 entries and p142_ceno_J2315, 7525 entries) comprising annotated proteins of various *Burkholderia* species (*Burkholderia cenocepacia* J2315, *Burkholderia cenocepacia* AU 1054, *Burkholderia cenocepacia* HI2424, *Burkholderia cepacia* AMMD, *Burkholderia* sp. 383, *Burkholderia mallei* ATCC 23344, *Burkholderia mallei* NCTC 10229, *Burkholderia mallei* NCTC 10247, *Burkholderia mallei* SAVP1, *Burkholderia pseudomallei* 1106a, *Burkholderia pseudomallei* 1710b, *Burkholderia pseudomallei* 668, *Burkholderia pseudomallei* 305, *Burkholderia pseudomallei* K96243, *Burkholderia thailandensis* E264, *Burkholderia vietnamiensis* G4, *Burkholderia xenovorans* LB400, *Burkholderia ambifaria* MC40-6,

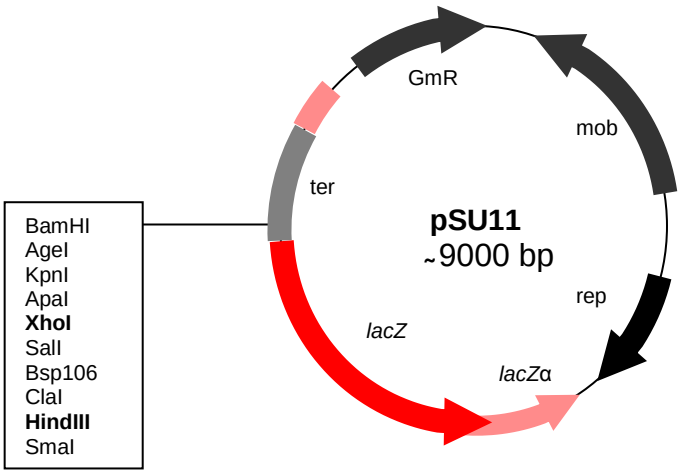
Burkholderia cenocepacia MC0-3, *Burkholderia multivorans* ATCC 17616, *Burkholderia phymatum* STM815, *Burkholderia phytofirmans* PsJN) and proteins of the closest relative of H111, *B. cenocepacia* J2315, respectively. Moreover, common contaminants such as trypsin and keratin were added to both of the databases to avoid false positive identification of proteins.

The ratio of peak areas between iTRAQ reporter ions 114, 115, 116 and 117 (analyses I and II) and reporter ions 117, 116, 115 and 114 (analyses III and IV) was used to determine the relative abundance of proteins in each protein sample. For normalization the ratio of each protein sample from iTRAQ analyses I or II and III or IV were multiplied. The root of the product was then extracted and further referred to as "normalized". The mean, standard deviation, and p-values to estimate statistical significance of protein quantification were calculated by the Mascot software. Proteins were assigned as QS-regulated when the regulation factors were higher than 1.5 in both independent analyses of either EC and/or WC proteins of the *cepR* and/or *cepI* mutant and the corresponding p-values were below 0.05.

2.4 Phenotypical assays

2.4.1 Analyses of gene expression measuring β-galactosidase activity

This assay was used to quantify promoter activities of genes by analysing the activity of a promoter-fused reporter gene, in this case the enzyme β-galactosidase. The analysis was conducted with plasmid pSU11 or pRN3 (2.1.1, see plasmid map below and dissertation S. Uehlinger, 2009). Plasmid pSU11 expresses the *E. coli lacZ* gene and comprises a multiple cloning side (*Xho*I and *Hind*III are preferentially used for ligation of DNA sequences) and a *mob* gene for plasmid mobilisation. The reporter activity was determined by the spectrometrically measurement of the product ONP (Ortho-nitrophenol, absorption wavelenght 420 nm) as described by Miller (Miller, 1972).



Overnight cultures of strains (grown in LB-medium supplemented with appropriate antibiotic or minimal medium) containing the transcriptional promoter-*lacZ* fusion were inoculated in the appropriate medium (OD_{600} of 0.01) and aliquots were withdrawn during bacterial growth. Volumes of aliquots were varying between 200 – 800 µl depending on the expected promoter activities. At each time point, the adsorbance at 600 nm was recorded and bacterial cells were pelleted for storage (-20°C) or for the subsequent analysis of promoter activity resuspended in 1 ml of Z-buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol, pH 7.0). Cell membranes were permealized by the addition of either toluene or 0.1% SDS-solution and chloroform. When adding toluene (a drop), cell samples were further incubated at 30°C (overnight). The enzyme assay was performed at 30°C, started by the addition of 200 µl of ONPG (4 mg/ml, o-nitrophenyl-β-D-galactopyranoside, Sigma) and stopped when the solution turned yellow by supplying 0.5 ml of 1 M Na_2CO_3. The reaction time was recorded, cell debris were removed by centrifugation, and absorbance was measured at 420 nm. Specific Miller units were calculated as follows: 1000 x OD420/ (t x V x OD_{600}), with t = reaction time and V = culture volume. Cell membranes were permealized with SDS and chloroform by the addition of each 20 µl to the samples, followed by vortexing and incubation of the samples for 8 min. Further steps of the assay were then performed as described above.

2.4.2 Extraction and detection of acyl-homoserine lactone (AHL) molecules

2.4.2.1 Detection of AHL molecules employing sensor plasmids

The detection of acyl-homoserine lactone (AHL) molecules was accomplished employing bacterial reporter systems (2.1.1. and see below). These systems differ in the type of reporter and in their detection specificity of AHL molecules.

***Escherichia coli* MT102 (pSB403):** bioluminescent AHL sensor plasmid; pRK415 plasmid backbone with *luxRI*::*luxCDABE* transcriptional fusion (Winsor *et al*., 1998). (*Vibrio fischeri luxR* gene and transcriptional fusion of *Vibrio fischeri luxI* promoter to *luxCDABE* of *Photorhabdus luminescens*. Reporter is most sensitive to 3-oxo-C6-HSL.)

***Pseudomonas putida* F117 (pAS-C8):** gfp-based AHL sensor plasmid; pBBR1MCS-5 plasmid backbone with P_{cepI}::*gfp*(ASV) P_{lac}::*cepR* (Steidle *et al*., 2001, Riedel *et al*., 2001). (*B. cenocepacia* H111 *cepR* gene under control of the P_{lac} and transcriptional fusion of H111 P_{cepI} to *gfp*(ASV). Reporter most sensitive for C8-HSL).

***Pseudomonas putida* (pKR-C12):** *gfp*-based sensor plasmid, pBBR1MCS-5 plasmid backbone with P_{lasB}::*gfp*(ASV) P_{lac}::*lasR* (Riedel *et al*., 2001). (*P. aeruginosa* PAO1 *lasR* gene under control of P_{lac} and transcriptional fusion of P_{lasB} to *gfp*(ASV). Reporter highly sensitive to 3-oxo-C12-HSL and 3-oxo-C10-HSL).

***Chromobacterium violaceum* CV026**: reporter based on violacein production, *C. violaceum* ATCC 31532 derivative, *cviI*::Tn5 (McClean *et al*., 1997). The production of the violet pigment violacein is positively-regulated via quorum sensing in *C. violaceum*. Mutant CV026 has a mutation in the *luxI* homologous gene *cviI* and produces violacein only when AHL molecules are externally added. This sensor is highly sensitive to unsubstituted short-chain AHL molecules but quite insensitive to other AHLs (McClean *et al*., 1997).

***E. coli* MT102 pJBA89:** *gfp*-based sensor plasmid, pUC18Not-*luxR*-P_{luxI}-RBSII-*gfp*(ASV)-T0-T1 (Andersen *et al*., 1998).

***E. coli* MT102 pJBA89*luxR*$^-$:** *luxR*-deficient derivate of pJBA89, pUC18Not-P_{luxI}-RBSII-*gfp*(ASV)-T0-T1 (see 2.2.6 and (Malott *et al*., 2009).

2.4.2.2 Qualitative and quantitative detection of AHL molecules

For the qualitative detection of AHL molecules, test strains were streaked on LB-agar plates in a 90° angle to a sensor strain without contact ("cross streaking"). The plates were incubated (1-3 days, at 30°C and 37°C) and analysed for reporter activity. The expression of GFP (green fluorescent protein) was monitored in a dark box by illumination with blue light (HQ 480/80 filter F44-001; AHF-Analysentechnik, Tübingen, Germany, light source Intralux 5000-1 halogen lamp; Volpi, Schlieren, Switzerland). The expression of bioluminescence was determined in a dark box and documented with a light-sensitive camera (Hamamatsu Photonics, Herrsching, Germany).

The quantity of AHL molecules was measured by detecting the reporter activity of sensor plasmids grown in wells of microtitre plates (FluoroNunc Polysorp microtiter plate (Nunc, Roskilde, Danmark). To analyse promoter binding of CepR2 we performed assays using sensor plasmids pJBA89 and pJBA89*luxR*$^-$ respectively, for the detection of AHL production characterizing the RsaM protein we employed sensor pAS-C8 (see below). In all cases, GFP-expression was analysed by determining the relative fluorescence units (RFU) using a microtiter reader (Synergy HT; Bio-Tek, Bad Friedrichshall, Germany) with excitation

wavelength of 485 nm and detection of emission at 528 nm. In all assays background fluorescence (sensor strain with LB-medium) was subtracted and data were plotted as relative fluorescent units (RFU).

2.4.2.3 Analyses employing reporter strains pJBA89 and pJBA89luxR$^-$

Overnight cultures of *E. coli* MT102 harboring plasmid pJBA89 or pJBA89luxR$^-$ were subcultured in 50 ml LB-medium in the presence or absence of plasmid pJTR2 (2.1.1) containing appropriate antibiotics to an OD_{600} of 0.1. At an OD_{600} of 1.5, cells were distributed in 200 µl aliquots into wells of a microtiter plate as described above. The wells contained no HSL or C14-HSL, 3-oxo-C12-HSL, C12-HSL, 3-oxo-C10-HSL, C10-HSL, 3-oxo-C8-HSL, C8-HSL, 3-oxo-C6-HSL, C6-HSL, or C4-HSL at final concentrations of 1500, 750, 375, 187.5, 93.8, 46.9, and 23.4 nM. After incubation of the plates at 30°C for 6 h, the green fluorescence of the sensor strains was measured as stated above.

2.4.2.4 Analyses of AHL production throughout the growth curve

For the characterization of RsaM overnight cultures of strains were washed and resuspended in 200 µl LB-medium to an OD_{600} of 0.05. Cells of exponentially-phase-grown *Pseudomonas putida* F117 (pAS-C8) were added obtaining final volumes of 300 µl. Samples were transferred to wells of microtitre dishes and incubated for 48h hours at 30°C. Expression of GFP was measured every hour and data were plotted as described above.

2.4.2.5 Extraction of AHL molecules and detection by thin layer chromatography

Acyl-homoserine lactone (AHL) molecules were extracted from supernatants of 100 ml overnight grown bacterial cultures in LB-medium. Briefly, cell cultures were centrifuged (5000 rpm, 20 min, 4°C), supernatants were acidified with 1 M HCL to yield pH 2.0 and transferred to a separating funnel. AHL molecules were extracted twice from organic phases by the addition of each 50 ml dichloromethane. The removal of remaining water was achieved by the addition of dehydrated $MgSO_4$ to the collected dichloromethane extracts. After filtering the solution through whatman paper, extracts were dried on a rotary evaporator using vaccum and the remainder (ca. 400 µl) was resolved in 200 µl of acidified ethyl acetate. AHL extracts were kept at -20°C and analysed by reverse-phase thin layer chromatography (TLC) as described in the following: 10 µl of AHL extracts and 1 µl of AHL standards [1 mM] were dropped on RP-TLC plates (RP-18 F254s, 20x20cm, Merck) and compounds were separated using a mobile phase with 60% methanol in dH_20. The detection of AHL molecules was

achieved by overlaying the dried plates with LB soft agar (2.1.2) containing 10 ml of exponentially-phase sensor strains (see above). Following an incubation period for 24 - 48h at 30°C, plates were examined for the extent of reporter activity as previously described.

2.4.3 Detection of extracellular protease activity

For the detection of extracellular protease production, 5 µl of liquid overnight cultures were spotted on milk-agar plates (2.1.2). The plates were then incubated at 37°C for 24 to 48h. Proteases activity was determined by measuring the clearing zones surrounding bacterial colonies.

2.4.4 Extraction and detection of siderophores

2.4.4.1 Extraction of pyochelin

The siderophore pyochelin was extracted from supernatants of bacterial cultures grown in succinate medium (2.1.2). For that purpose, overnight cultures were initially incubated in 50 ml succinate medium to an OD_{600} of 0.05 and grown with agitation for 40h at 37°C. The cells were pelleted (5000 rpm, 20 min, 4°C), the collected supernatant was acidified for the stabilization of the siderophore (addition of 1M HCL to yield pH 2.0) and transferred to a separating funnel. Pyochelin was extracted with 20 ml of dichloromethane (twice), organic phases were collected and exposed to dehydrated $MgSO_4$ in order to remove remaining water. Filtered extracts were dried on a rotary evaporator to about 50 - 100 µl, then resuspended in 100 – 200 µl of methanol. For further concentration pyochelin extracts were dried (speed-vac or at 42°C in a heating block) and resuspended in a final volume of 20 µl of methanol. Pyochelin extracts were stored at -20°C and later on analysed employing CAS plates or by thin layer chromatography (2.4.4.3).

2.4.4.2 Extraction of ornibaction

Ornibactin was extracted from supernatants of 50 ml bacterial cultures grown in succinate medium as described for the extraction of pyochelin except that the culture media were amended with ornithine [10 mM L-ornithine dihydrochloride]. The cells were pelleted (5000 rpm, 20 min, 4°C) and the supernatant acidified for the stabilization of the siderophore as described, then transferred to a separating funnel. Ornibactin was extracted with 20 ml of dichloromethane (twice) from aquarous phases, which were collected and dried on a rotary evaporator to about 50 - 100 µl using vacuum and heating (40°C). Extracts were resuspended in 100 – 1000 µl of methanol and transferred to a collection tube (salt precipitations were

transferred along). After centrifugation (8000 rpm, 10 min, 4°C) and transfer of the supernatant to a new collection tube, the pellet was again resuspended in methanol (200 – 500 µl) to remove the remaining ornibactin. Samples were again centrifuged (8000 rpm, 10 min, 4°C), the supernatant was combined with the one collected at the beginning and ornibactin extracts were further dried on a rotary evaporator to a final volume of 1 ml. Extracts were stored at -20°C and later on analysed using CAS plates or by thin layer chromatography (2.4.4.3).

2.4.4.3 Detection of siderophores on CAS plates and by thin layer chromatography

The production of siderophores was determined employing the chrome azurol S (CAS) assay (Schwyn, 1987) with the following modifications: agar was prepared without MM9 buffer and contained LB-medium instead of casamino acids as a carbon source. When iron is removed from the original CAS-Fe(III) complex during siderophore production blue-to-orange halos surround bacterial colonies. Formation of halos is then evaluated following 1 or 2 days of plate incubation at 37°C. The assay was employed for the analysis of siderophores produced by bacterial colonies grown on CAS plates or siderophore extracts spotted on the agar. In each case, a volume of 5 µl (overnight cultures of bacterial strains grown in LB-medium or siderophore extracts, see before) was dropped.

CAS-agar plates were made by freshly preparing solution 1 and solution 2 (see below). 10 ml of the Fe^{3+}-solution were gently added to solution 1, then solution 2 was added to obtain the chromazurol S/ Fe^{3+}/HDTMA complex (notice a change in colour from red to violet). For the preparation of LB-PIPES, PIPES was first dissolved in dH_2O and stired while supplying NaOH [10M] until the solution became clear. The remaining components for LB-PIPES (see below) were added and the pH of the solution was titrated to pH 6.8. After autoclaving both solutions, the chromazurol S/ Fe^{3+}/HDTMA complex solution and the LB-PIPES agar, the former complex solution was poured into the LB-PIPES agar, shortly mixed and used for the preparation of CAS plates.

Solution 1		Solution 2	
Chromazurol S	60.5 mg	HDTMA	72.9 mg
ddH_2O	ad 50 ml	ddH_2O	ad 40 ml

Fe^{3+}-Solution		LB-PIPES agar	
$FeCl_3$	1 mM	PIPES (Sigma)	30.24 g
HCl	10 mM	Bacto tryptone	10.00 g
ddH_2O	ad 100 ml	Bacto yeast extract	5.00 g
		NaCl	4.00 g
		Agar	12.00g
		ddH_2O	ad 900 ml

Siderophore extracts were also analysed employing thin layer chromatography (TLC, see J. Toller, Master thesis, 2008, for a detailed description). Briefly, 10 - 15 µl of siderophore extracts were spotted on TLC silica plates 60 F_{254} (Merck, Darmstadt, Germany), and compounds were separated using a mobile phase with chloroform: acetic acid: ethanol (90:5:2.5) (Cox & Graham, 1979, Visca *et al.*, 1992). Salicylic acid (7 µl of a 500 mM stock solution) was dropped on the plates as a standard. Plates were analysed under UV light (254 nm, 366 nm, green filter (Cosmicar Pentax, CCTV Camera Lens). Siderophores were also detected by spraying an iron reagent solution (0.1 M $FeCl_3$ in 0.1 M HCl) on the TLC plates (Visca *et al*., 1992).

2.4.5 Analysis of bacterial motility

The ability to swarm or swim was assayed on swarming or swimming agar plates (see 2.1.2) at RT, 30°C and 37°C. The conditions used were those found to be best for the analyses of bacterial strains. For H111 these conditions were an incubation period at 30°C for 48h.

2.4.6 Analysis of biofilm formation

2.4.6.1 Biofilm formation under static conditions in microtitre plates

Biofilm development in polystyrene microtitre plates (Sarstedt, Newton, USA) was assayed as described (O'Toole & Kolter, 1998) and (Huber *et al*., 2001) after an incubation period of 48h at 30^{o}C. Biofilms were grown in AB media supplemented with either 10 mM citrate or 10 mM D-glucose (2.1.2). The media was also adjusted to pH 7 with 100 mM phosphate buffer.

2.4.6.2 Biofilm formation under dynamic conditions in artificial flow chambers

To monitor biofilm formation under dynamic conditions, strains were genetically tagged with the green fluorescent protein (GFP) encoded on plasmid pBAH8 (2.1.1), and biofilms were grown at 30°C in artificial flow-through chambers (see below) supplied with FAB-medium containing 1 mM D-glucose (2.1.2).

Set-up and sterilization of the biofilm system

The system was assembled as previously described (Christensen *et al.*, 1999). Prior inoculation of flow chambers with bacteria the channels were sterilised with 0.5% hypochloride (at least 3 - 4 h), followed by a washing step with dH_20 overnight. The bacterial growth medium (FAB-medium supplemented with 1 mM D-glucose, 2.1.2) was then pumped at a constant flow rate of 0.2 mm/s with a Watson-Marlow 205S peristaltic pump through the system.

Inoculation of flow chambers, growth and analyses of biofilms

Overnight cultures of bacterial strains (grown in LB-medium) were inoculated in FAB-medium to an OD_{600} of 0.1. Approximately 800 µl of this suspension were then injected into sterile flow chambers employing a syringe. For the initial attachment of bacteria onto the glas slide the flow chambers were inverted and incubated for one hour at RT, then again flipped and exposed to a constant medium flow at a rate of 0.5 mm/s using a Watson-Marlow 205S peristaltic pump. Biofilms were grown at 30°C and inspected microscopically (12h to 96h) with a confocal laser scanning microscope (Leica, DM 5500). Data were analysed with Leica Application Suite (Mannheim, Germany) and Imaris software package (Bitplane, Switzerland). Images were prepared for publication using Photoshop CS (Adobe).

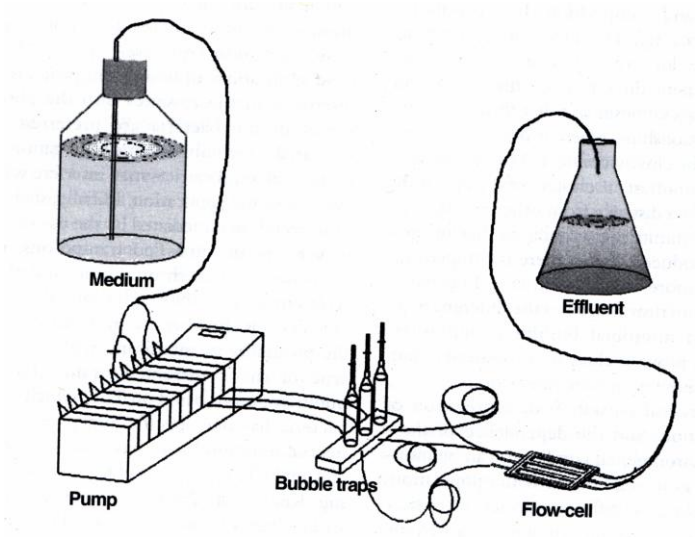


Fig. 3. System set-up of biofilms (Jan Molin, 2000).

2.4.6.3 Visualization of dead bacteria by staining with propidiume iodide

Dead cells were visualized by cellular staining (20 - 30 min, flow rate as above) with 0.5 µM propidium iodide (Fluka, Buchs, Switzerland) in FAB-medium (2.1.2 for propidiume iodide stock solutions). Fluorescence was detected by confocal laser scanning microscopy (Leica, DM 5500 Q) with laserline 532.

2.4.7 Metabolic profiling employing BIOLOG microarrays

BIOLOG phenotypic microarray system consists of 96 well microtiter plates containing minimal medium supplemented with varying carbon (PM1), nitrogen (PM2), sulfur (PM3) and phosphorus sources (PM4). The assay uses a reporter which monitors cell respiration reflecting the bacterial growth. If the phenotype is strongly "positive" in a well, the cells respire actively and reduce a tetrazolium dye, which results in the development of a strong color (blue to violet). If it is weakly positive or negative, respiration is slowed or stopped, and less color or no color is visible. The exact formulation of the media is proprietary of BIOLOG, Inc. (Hayward, USA), and was moreover described by Bochner and collegues (Bochner *et al.*, 2001). Minimal medium used in the study contained 100 mM NaCl, 30 mM triethanolamine HCI (pH 7.1), 25 mM sodium pyruvate, 5 mM NH_4Cl, 2 mM NaH_2PO_4, 0.25 mM Na_2SO_4, 0.05 mM $MgCl_2$, 1 mM KCl, 1 mM $FeCl_3$, and 0.01% tetrazolium violet.

The assay was performed following the protocol of BIOLOG, Inc. (see S. Uehlinger, Dissertation, 2009) as followed: strains were grown overnight on R2A agar (Becton-Dickinson) and resuspended in IF-0 inoculation fluid corresponding to the turbidity of the 85%T turbidity standard (both supplied by the company). After addition of the tetrazolium redox dye (supplied by the company), the suspension was distributed in volumes of 100 µl to the wells of the microtiter plates. For PM 3 and 4, an extra supplementation with a carbon source was required, consisting of 20 mM sodium succinate and of 2 µM ferric citrate. The plates were incubated at 37°C for 24 hours and scanned for visual analysis.

2.4.8 Pathogenicity model experiments

2.4.8.1 The *Caenorhabditis elegans* infection model

Caenorhabditis elegans slow-killing assays were performed as described by Koethe and co-workers (Köthe *et al.*, 2003). In this assay larvae of *C. elegans* Bristol N2 (nematodes were obtained from the *Caenorhabditis* Genetics Centre, University of Minnesota, USA) are fed on bacterial lawns, which results in the bacterial colonization of the intestine followed by nematode death over a period of 2 to 3 days. Overnight cultures of bacterial strains were

plated on 6-well plates containing NGM II nematode growth medium (51 mM NaCl, 0.35% (w/v) bactopeptone, 1.7% agar, (Tan *et al.*, 1999) with supplements (25 mM KPO_4 buffer, pH 6, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 2 µg/ml uracil, 5 µg/ml cholesterol, 50 µg/ml nystatine solution). Buffers and stock solutions are listed below. The plates were incubated at 37°C for 24h. Approximately 20 to 40 hypochlorite-synchronized larvae (see egg preparation) were then dropped on bacterial lawns, followed by incubation of the plates at 20°C. Worms were counted after an incubation period of 24h, 48h and 72h using a Leica M165FC stereomicroscope. *C. elegans* were cultivated on NGM I nematode growth medium (51 mM NaCl, 0.25% (w/v) tryptone, 1.7% (w/v) agar with supplements (see above,(Hope, 1999) at 20°C with *E. coli* strain OP50 (2.1.1) as a food source. For the cultivation of *C. elegans* small agar pieces with nematodes were transferred onto fresh *E. coli* lawns on NGM I plates once a week.

Nystatin-solution: 10 mg/ml in 100% ethanol and 7.5 M ammonium acetate
KPO4 buffer: 0.8 M KH_2PO_4, 0.2 M K_2HPO_4, pH 6.0
M9 buffer: 22 mM KH_2PO_4, 42 mM Na_2HPO_4, 85 mM NaCl, 1 mM $MgSO_4$

Cholestrol and Uracil stock solutions: see 2.1.2. NGM I growth medium was filled in Petri dishes (5.5 cm in diameter, Greiner, Frickenhausen, Germany), NGM II growth medium was poured into 6 well plates (4 ml/well, Greiner, Frickenhausen, Germany).

Egg preparation for the synchronization of *C. elegans*

All pathogenicity assays were performed with a synchronized *C. elegans* population of L4 stage worms. For that purpose nematode eggs were separated from adult worms using the following bleaching method: NGMI-agar plates that contained a large number of eggs were rinsed four times with sterile water, and the suspension was distributed (each ca. 1ml) into three 2 ml collection tubes. 500 µl of bleaching solution (600 µl sterile MQ water, 500 µl of 12% sodium hypochlorite, 400 µl 6 M NaOH) was added to each tube and tubes were incubated for 8 to ca. 10 min and occassionally inverted. After centrifugation (3200 rpm, 1 min) pellets were washed with sterile water and the suspension was again centrifuged, and pellets were resuspended and combined in a final volume of 100 µl of M9 buffer (22 mM KH_2PO_4, 42 mM Na_2HPO_4, 85 mM NaCl, 1 mM $MgSO_4$). The eggs were transferred to a NGM I-agar plate containing *E. coli* OP50 and incubated for the development to L4-larvae at 20°C for 48h.

2.4.8.2 *Galleria mellonella* killing assay

As an alternative model organism to study the pathogenicity of *Burkholderia* strains, the larvae of the greater wax moth *G. mellonella* were used. The assay was done as described by Uehlinger and collegues (Uehlinger *et al.*, 2009) and (Jander *et al.*, 2000, Seed & Dennis, 2008). *G. mellonella* caterpillars in the fifth instar were obtained in fishing equipment stores (Brumann, Zürich or Hebeisen, Zürich) and stored at 15°C. They were used no later than 2 to 3 weeks from the date of purchase. Bacterial overnight cultures grown in LB broth were diluted 1:100 in 30 ml of the same medium and cultivated to an optical density at 600 nm (OD_{600}) of 0.4 to 0.7 (logarithmic growth phase). Cultures were centrifuged, and pellets were resuspended in 10 mM $MgSO_4$ to an OD_{600} of 1. Four 2-fold dilutions were prepared in 10 mM MgSO4, resulting in dilutions of OD_{600} of 0.5, 0.25, 0.125 and 0.0625. To determine the CFU per injection volume, serial dilutions were made and aliquots were plated on LB agar. Colonies were counted and the number of bacteria was calculated. A 1 ml syringe (BD Plastipak, Madrid, Spain) with a 27G x 7/8" needle (Rose GmbH, Trier, Germany) was used to inject a 10 µl aliquot into *G. mellonella* via the hindmost proleg. The injection area was previously disinfected with a cotton swab soaked in ethanol. Ten to fourteen healthy, randomly chosen larvae were injected per strain and dilution and incubated in Petri dishes (in the dark, 30°C). A control injection with 10 µl of 10 mM $MgSO_4$ that contained antibiotics that were used to grow bacterial cultures was performed to monitor killing of animals due to physical injury or infection by contaminating pathogens. Likewise, the toxicity of an antibiotic compound was excluded. The number of dead larvae was scored 24, 48 and 72 h after infection. Dead larvae showed discoloration due to melanization and did not display movement in response to touch. Experiments with more than one dead larva in the control group were not considered and repeated. Experiments were repeated at least three times.

3 Results

3.1 Project I: The *Burkholderia cenocepacia* H111 quorum sensing regulon: a comparative transcriptomic, proteomic and phenotypic analysis

This project aimed at the identification of genes and proteins that are regulated by the CepIR quorum sensing (QS) system in *B. cenocepacia* H111. For this purpose, the H111 QS regulon was mapped employing a combination of the following approaches: we performed two-color microarray analyses, proteome studies employing iTRAQ (isobaric tag for relative and absolute quantitation), and experiments using phenotypic microarrays, where the importance of QS for the utilization of various substrates was examined. Our investigations led to the identification of known and potential virulence factors as well as functions that may contribute to bacterial adhesion and biofilm formation.

3.1.1 Mapping the CepR regulon of *B. cenocepacia* H111 by transcriptomics

To identify CepR-regulated genes in *B. cenocepacia* H111 we compared the transcriptome of the wild type strain with the one of the *cepR* mutant H111-R using a custom *B. cenocepacia* oligonucleotide microarray. As a control we included the complemented *cepR* mutant H111-R (pBAH27) in these experiments. Bacterial strains were grown as described (2.2.1) and total RNA was extracted when the cultures had reached an OD_{600} of 2.5 (2.2.9). At this density transcription of *aidA*, a stringently CepR-regulated gene, was found to be maximal (Fig. A1, Appendix). Transcriptomic expression trends are as follows: From a starting set of 9622 genetic elements (CDSs, rRNA, tRNA and intergenetic regions) microarray analysis, using an initial filtering with an arbitrary cut-off value of 2-fold ratio change, revealed 156 genetic elements down- and 60 elements up-regulated in the transcriptome of H111-R, whereas transcript levels of 236 genetic elements were down- and 185 elements up-regulated in the transcriptome of H111-R (*cepR*$^+$), respectively (see Table A1 and A2, Appendix). For a more detailed analysis the most stringently CepR-activated genes, i.e. those 48 genes which exhibited a ≥3-fold reduction in transcript levels in the H111-R transcriptome, were chosen (Table 5). The transcriptome of strain H111-R (*cepR*$^+$) and the proteomes of mutant H111-R and H111-I were subsequently examined for the differential expression of these genes or proteins (Table 5). In fact, complementation of H111-R with *cepR* on plasmid pBAH27 rescued expression of genes in the mutant strain in most cases (Table 5 and Table A2). Notably, several of the CepR-activated genes were found to be even more strongly expressed in the complemented *cepR* mutant than in the wild type, probably due to the increased copy number of *cepR*. Some of these up-regulated genes were only slightly down-regulated in the

cepR mutant H111-R (Table 5 and Table A1/A2), suggesting that expression of these genes may be maximal at another time point during growth. Alternatively, activation of these genes may be the result of unspecific binding of CepR to operator sequences.

Several of the CepR-activated genes appear to be organized in operons, as they are transcribed in the same orientation and show similar levels of regulation. We therefore also visualized potential QS-activated operons that were identified in this study and report *cep* boxes that have been identified within promoter regions of these genes (Fig. 6).

3.1.2 Identification of QS-regulated proteins in *B. cenocepacia* H111

To confirm and extend our transcriptome analysis we compared the proteome of the wild type with the one of mutant H111-R and the proteomes of mutant H111-I grown in the presence or absence of 200 nM C8-HSL. Extracellular (EC) and whole cell (WC) protein fractions were extracted from cultures grown to an OD_{600} of 3.0, a culture density, at which AidA was maximally expressed as previously identified by Western blotting (data not shown). Only those proteins, which were identified in both of the two independently performed proteome analyses, and which were found to be at least 1.5-fold differentially expressed when wild type and mutant strains were compared (2.3.6) were considered as QS-regulated (Table A3, Appendix). Using iTRAQ coupled with tandem mass-spectrometry (MS/MS, 2.3.6) we identified 1257 protein in total, of which 218 proteins (17.3%) were up- and 210 proteins (16.7%) down-regulated in either the *cepR* and/or the *cepI* mutant relative to the wild type strain.

The proportional quantity of 1.5-fold up- or down-regulated EC or WC proteins in mutant H111-R and mutant H111-I is depictured in Fig. 4. Noticeable, the expression of a comparatively large number of proteins in the H111-R proteome seems to be up-regulated. As most of these proteins were identified in the EC protein fractions (Fig. 4), it might be speculated that the observed differences result from cell lysis, which might have occured during sample preparation.

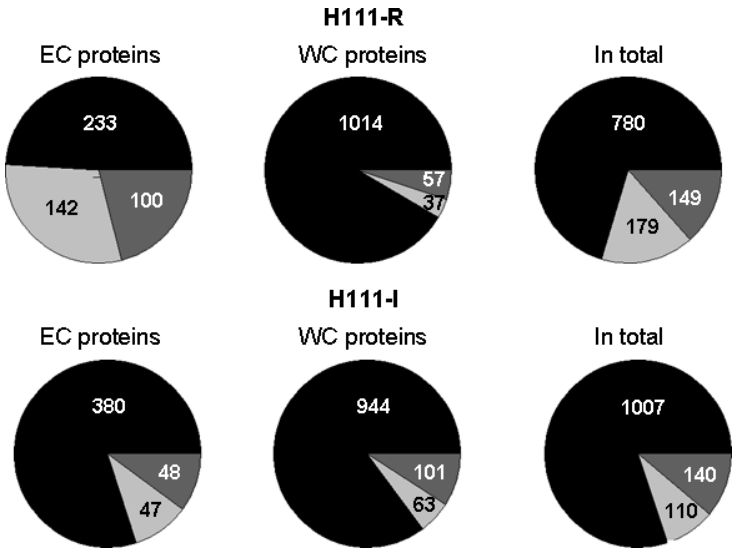


Fig. 4. Circular chart of QS-regulated proteins identified in two independent iTRAQ analyses. Depicted is the quantity of proteins identified in the *cepR* mutant H111-R or in the *cepI* mutant H111-I, with an unchanged (black background) or a more than 1.5-fold up-regulated (light grey background) or down-regulated (dark grey background) expression when compared to the proteome of the H111 wild type. EC: extra-cellular proteins; WC: whole cell proteins. Data are derived from two independent iTRAQ experiments.

3.1.3 Correlation between transcriptomic and proteomic data

Our study identified 18 genes encoding differentially expressed proteins (≥1.5-fold regulated in the proteomes of mutant H111-R and/or H111-I, Table A3, Appendix) which were also among the ≥2-fold regulated genes identified in the transcriptome analysis (Table A1/A2).

Transcriptomic expression trends compared to the quantity of proteins identified by iTRAQ are moreover shown in an overlap analyses of 3-fold differentially expressed genes or proteins in strains H111-R and H111-R (*cepR*$^{+}$) (Fig. 5). It must be noted that a direct comparison between QS-controlled genes and proteins should probably be taken with caution, since the microarray and iTRAQ analyses exhibit method-specific differences in the detection sensitivities and in the susceptibility to errors, as for instance the possible occurance of cell lysis during the extraction of EC proteins. Employing a cut-off of 3-fold ratio change for the proteome data of the *cepR* mutant, a threshold that was previously used to identify the most stringently controlled CepR-regulated genes (Table 5), 34 proteins (2.7%) reveal an up-regulated, and 13 proteins (1.0%) a reduced expression in the EC and/or WC protein fractions of the H111-R proteome (Fig. 5 and Table A3). Overall, we were able to identify an adequate proportion of ≥3-fold CepR-regulated genes or proteins of *B. cenocepacia* H111 employing transcriptomics (1.4%) or proteomics (3.7%).

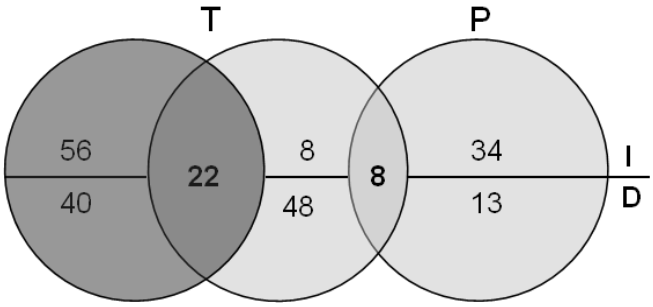


Fig. 5. Overlap analysis of 3-fold differentially expressed genes and gene products. Genes or proteins with a ≥3-fold differentially regulated expression in comparison to the wild type were identified in the transcriptomes (T) of mutant H111-R (light grey background) and mutant H111-R (*cepR*$^+$) (dark grey background), and in the proteome of the *cepR* mutant (P, EC and WC protein fractions). Numbers indicate the number of genes/proteins with increased (above the horizontal line; I) and decreased (below the horizontal line; D) expression. Bold numbers represent genes/proteins which were seen ≥3-fold positively regulated by CepR in the transcriptomes of mutant H111-R and H111-R (*cepR*$^+$) and in the EC and/or WC fractions of the H111-R proteome (see Table 5).

3.1.4 The H111 CepR-activated regulon identified by transcriptomics and proteomics

Genes or proteins, which comprise the H111 CepR-activated regulon, are listed in Table 5. They include those 48 genes, whose expression was seen most strongly induced by CepR (≥ 3-fold reduction of transcript levels in H111-R). Transcript levels of 32 out of these genes were, in turn, increased when *cepR* was provided in *trans* and protein amounts of 12 genes were also reduced in mutant H111-R or mutant H111-I as identified by iTRAQ analyses. Table 5 depicts genes which have been repeatedly reported as QS-controlled, including three well characterized virulence factors. These genes are: the zinc metalloproteases ZmpA (BCAS0409), (Sokol *et al*., 2003), (Chambers *et al.*, 2006), (Subsin *et al.*, 2007) and ZmpB (BCAM2307), (Riedel *et al*., 2003), (Kooi *et al.*, 2006) and the protein AidA (BCAS0293), (Riedel *et al*., 2003), (Huber *et al*., 2004), (Wopperer *et al*., 2006). While both proteases are thought to be important for the infection of mammalian hosts, it was recently shown that AidA exerts a high specificity for *C. elegans* pathogenicity (Uehlinger *et al*., 2009). Our data show that expression of AidA is in fact strongly dependent on an intact CepIR system and thus *aidA* exhibits the greatest changes of transcript levels in the microarray profiling (-167.2-fold) and its product is the most QS-induced protein in *B. cenocepacia* H111 (Table A3, Appendix).

Notably, 31 of the 48 listed CepR-induced genes (Table 5) have so far not been associated with cell density-dependent expression, even though several attempts have been undertaken to identify QS-regulated genes in *B. cenocepacia* species (for references see Table A1, Appendix). Our study identified 14 genes which have also been reported as QS-regulated in a recent transcriptome mapping of *B. cenocepacia* K56-2 using the same custom

oligonucleotide microarrays (O'Grady *et al*., 2009), (Table 5, indicated by bold type). *B. cenocepacia* QS-regulated genes, which were identified in this study but have not been described so far include the cable pilus associated adhesin protein *bapA* (BCAM2143) and the downstream genes BCAM2142-40, and *fimA* (BCAL1677) plus the adjacent genes BCAL1678-BCAL1681 (Table 5). Three more gene clusters seem to be under QS control as indicated by our transcriptomic and proteomic profiling (Table 5 and Fig. 6). They comprise the functionally unknown gene BCAL0834, the putative beta-lactamase BCAM0393, the LysR family regulatory protein BCAL3178, the putative D-lactate dehydrogenase BCAL3179, as well as BCAM2308, encoding a putative leucyl aminopeptidase precursor, located downstream of the QS-regulated gene *zmpB* (Kooi *et al*., 2006). To our knowledge, the expression of these genes has not previously been reported to be QS-regulated, indicating strain specific differences of the CepIR regulons of Bcc species. Other genes with a ≥3-fold decrease in expression levels which are unique for strain H111 include BCAL0121, BCAL0510, BCAL1813, BCAL1921, BCAL3285, BCAM0028 and BCAM0030, BCAM0835, BCAM2684, BCAM2720, BCAS0236 and BCAS0498 (Table 5, Table A1).

Gene no. [a]	**Description**	**Transcriptome** [b]	**Proteome** [c] **(EC and WC proteins)**	
		Fold change		
		H111-R	**H111-R (*cepR*$^+$)**	**H111-R and/or H111-I**
BCAL0121	aquaporin Z	-3.3 *	–	–
BCAL0358	metallo peptidase, family M1	-4.7	2.5	↓
BCAL0510	conserved hypothetical protein	-5.7	–	–
BCAL0831	putative storage protein	-3.2	2.3	↓
BCAL0833	putative Acetoacetyl-CoA reductase	-3.0	4.6	–
BCAL1677	putative type-1 fimbrial protein	-8.7	–	↓
BCAL1678	putative outer membrane usher protein precursor	-6.4	–	–
BCAL1679	putative fimbrial chaperone	-5.5	–	–
BCAL1680	putative type-1 fimbrial protein	-6.2	–	–
BCAL1681	putative exported protein	-8.6	–	–
BCAL1813	multidrug efflux system outer membrane protein	-5.3 *	4.1 *	–
BCAL1921	conserved hypothetical protein	-3.5	–	–
BCAL3178	LysR family regulatory protein	-5.0 *	–	↓
BCAL3179	probable D-lactate dehydrogenase	-3.4 *	2.6	–
BCAL3285	Flavohemoprotein	-25.8	–	–
BCAM0028	putative FHA-domain protein	-5.1	–	–
BCAM0030	conserved hypothetical protein	-3.1	4.2 *	–
BCAM0184	BclC lectin	-5.1 *	3.3	↓
BCAM0185	BclB lectin	-3.3 *	–	–
BCAM0186	BclA lectin	-9.0	5.1 *	↓
BCAM0188	N-acylhomoserine lactone dependent regulatory	-4.1 *	4.5 *	–
BCAM0189	AraC family regulatory protein	-3.9	2.4	–
BCAM0190	putative aminotransferase – class III	-22.1 *	6.0	–
BCAM0191	putative non-ribosomal peptide synthetase	-12.8 *	3.5 *	–

BCAM0192	conserved hypothetical protein	-46.8 *	6.2	–
BCAM0193	conserved hypothetical protein	-47.7 *	6.8	–
BCAM0194	conserved hypothetical protein	-52.2 *	8.4	–
BCAM0195	putative non-ribosomal peptide synthetase	-34.0 *	6.9 *	–
BCAM0196	conserved hypothetical protein	-37.7 *	6.3 *	–
BCAM0200	efflux system transport protein	-9.2 *	15.0 *	–
BCAM0392	putative acetyltransferase	-3.8 *	3.9 *	–
BCAM0393	putative beta-lactamase, class D	-4.1 *	5.6 *	–
BCAM0835	AraC family regulatory protein	-4.3	3.5	–
BCAM1869	conserved hypothetical protein	-6.1 *	–	–
BCAM1870	N-acylhomoserine lactone synthase CepI	-27.0 *	–	–
BCAM1871	conserved hypothetical protein	-32.5 *	2.3	–
BCAM2140	transporter system transport protein	-3.1 *	2.4	–
BCAM2141	ABC transporter ATP-binding membrane protein	-4.4 *	–	–
BCAM2142	transport system outer membrane protein	-5.3 *	–	–
BCAM2143	cable pilus associated adhesin protein	-5.1 *	2.6	↓
BCAM2307	zinc metalloprotease ZmpB	-8.7 *	6.8	↓
BCAM2308	putative leucyl aminopeptidase precursor	-5.3 *	5.7	↓
BCAM2720	putative phospholipase C	-4.4	-2.3	–
BCAS0236	putative haemagglutinin-related autotransporter	-5.4	-2.1	–
BCAS0292	conserved hypothetical protein	-138.1 *	7.8 *	↓
BCAS0293	nematocidal protein AidA	-167.2 *	24.0 *	↓
BCAS0409	zinc metalloprotease ZmpA	-4.4	3.5	↓
BCAS0498	muconate cycloisomerase I 2	-4.9	-2.4	–

Table 5. CepR-induced genes and proteins. Listed are genes and ORFs (nomenclature according to Holden *et al*. (2009); a), whose expression is at least 3-fold reduced in the *B. cenocepacia* H111-R transcriptome relative to the wild type; b. Some genes are also found to be overexpressed in the complemented mutant H111-R (*cepR*$^{+}$). A corresponding decrease in extracellular (EC) or whole cell (WC) proteins in the H111-R and H111-I proteome is indicated by an arrow (c, see Table A3, Appendix, for iTRAQ data). P-values (<0.05) of significantly regulated genes are indicated by an asterisk. *B. cenocepacia* K56-2 QS-regulated genes which were identified in a previous study are displayed in bold type (O'Grady *et al*., 2009). QS-regulated factors of Bcc species which were identified by alternative methods are highlighted in grey (see Table A1 for references).

The CepIR system is autoregulated in that CepR positively regulates the expression of the *cepI* gene (Lewenza *et al*., 1999) and accordingly the transcript levels of *cepI* were found to be reduced in mutant strain H111-R (Table 5, Fig. 6). Interestingly, transcription of the adjacent genes BCAM1869 (-6.1-fold) and BCAM1871 (-32.5-fold) was also diminished in the H111-R mutant. This result is in full agreement with the study of O'Grady and colleagues (O'Grady *et al*., 2009). Both genes BCAM1869 and BCAM1871 are conserved among Bcc species but their functions remain to be elucidated (Winsor *et al*., 2008).

Other genes, whose expression was found to be stimulated by CepR in both *B. cenocepacia* strains H111 (≥ 2-fold reduced transcript levels in H111-R) and K56-2 (O'Grady *et al*., 2009), (see Table A1/A2, Appendix, genes in bold type) include the cold shock-like protein CspA (BCAL3006), the chitinase BCAL1722 (Huber *et al*., 2001), (-2.1-fold reduced in the H111-R transcriptome) and the putative lipoprotein BCAL1520 (-2.0-fold reduced in the H111-R transcriptome). Interestingly, transcript levels of *shvR* (BCAS0225), encoding a regulator involved in a switch from rought to shiny colony morphology (O'Grady *et al*., 2009), were

also deminished in strain H111-R (-2.4-fold). However, in contrast to the study by O'Grady and colleagues, we did not observe any QS regulation of the genes located in the vicinity of *shvR* (Table A1). Among other interesting CepR-regulated genes that were identified in the transcriptome analysis were BCAM2340 and BCAM2338, encoding proteins with a potential function in the synthesis of rhamnolipids (Table A1).

Remarkably, the transcription of genes encoding QS-controlled proteins identified by iTRAQ was in many cases not differentially regulated as seen in the microarray profiling (Table A3, Appendix). It is therefore reasonable to assume that at least some of these proteins are modulated at the posttranscriptional level by QS circuitry, as it was suggested in a previous study on the *P. aeruginosa* QS-regulated proteome (Arevalo-Ferro C & K, 2003).

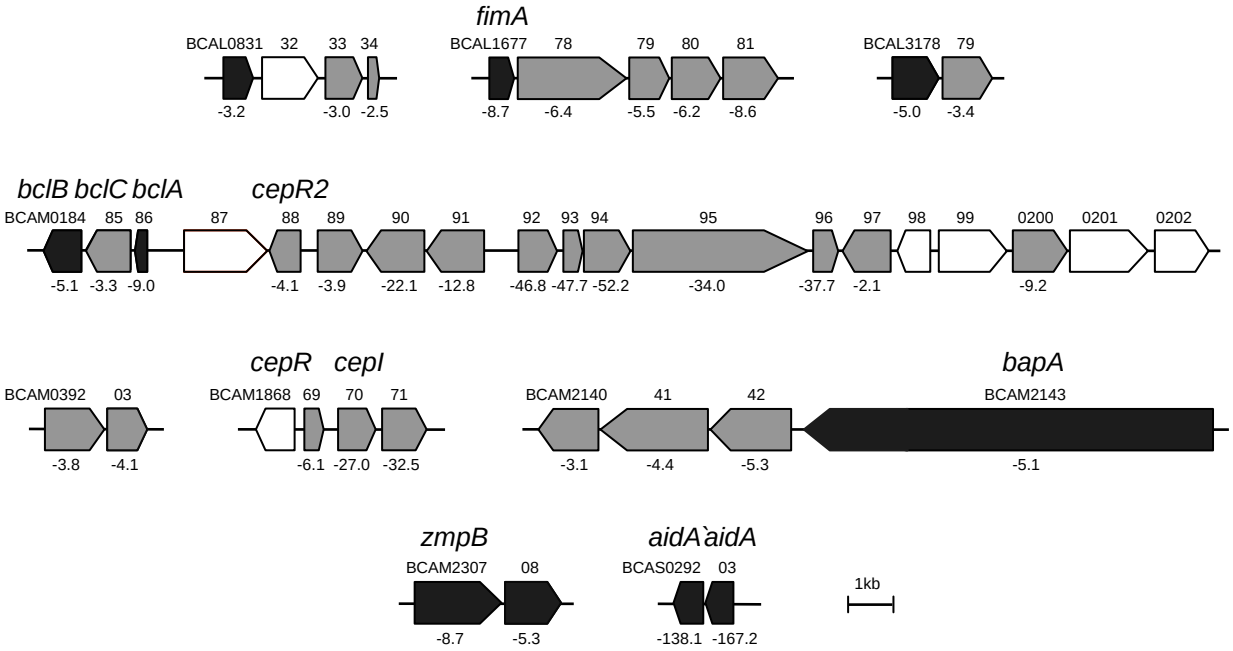


Fig. 6. Predicted QS-regulated operons in *B. cenocepacia* H111. Arrows indicate the direction of transcription and fold change-values (H111-R transcriptome) are indicated below individual genes. Genes in light grey were differentially expressed as seen in the microarray data, the expression of genes in dark grey was observed to be under QS control in both transcriptome and proteome analyses. Numbers refer to *B. cenocepacia* J2315 annotated genes (Holden *et al*., 2009). Previously described *cep* boxes were reported to be present upstream of BCAL0831 and BCAM1868 (Chambers *et al*., 2006), BCAM1870 (CCCTGTAAGAGTTACCAGTT, Huber *et al*., 2001), BCAM0189 (Malott *et al*., 2009) and BCAS0293 (Huber *et al*., 2004).

The following three QS-activated loci (see Table 5 and Fig. 6) were further characterized in this work: the orphan LuxR homologous transcriptional regulator CepR2 (BCAM0188, see 3.2), the BclACB lectins (BCAM0184-186, see 3.3), and BCAM1869, a protein of unknown function (see 3.4). As previous work has demonstrated that biofilm formation of *B. cenocepacia* H111 is regulated by a cell density-dependent expression (Huber *et al*., 2001),

our intention was also to identify QS-regulated factors that would potentially link QS and biofilm formation. Here, we focused on analysing the contribution of the BclACB proteins to the development of bacterial biofilms (see 3.3).

3.1.5 Overexpression of CepR leads to reduced expression of motility-associated genes

Previously, we observed that transcription of some of the CepR-regulated genes was only slightly altered in mutant H111-R but greatly affected in the *cepR* complemented strain H111-R (*cepR*$^+$). For that reason, we hypothesized that gene expression in *B. cenocepacia* H111 might be dependend on the cellular amount of the CepR protein. This regulatory trend becomes apparent when analysing the expression changes of motility-associated genes: transcript levels of 39 genes encoding proteins involved in flagellar biosynthesis, assembly or chemotaxis were down-regulated in strain H111-R (*cepR*$^+$) (Table A2, Appendix). By contrast, transcription of only one of these genes, *flhD* (BCAL0124), which encodes the flagellar master regulator was differentially expressed in the transcriptome of mutant H111-R (2.2-fold up-regulated, Table A1).

The investigation of swimming motility of the H111 wild type and QS mutant strains revealed a negative effect of the CepIR system (Fig. 7).

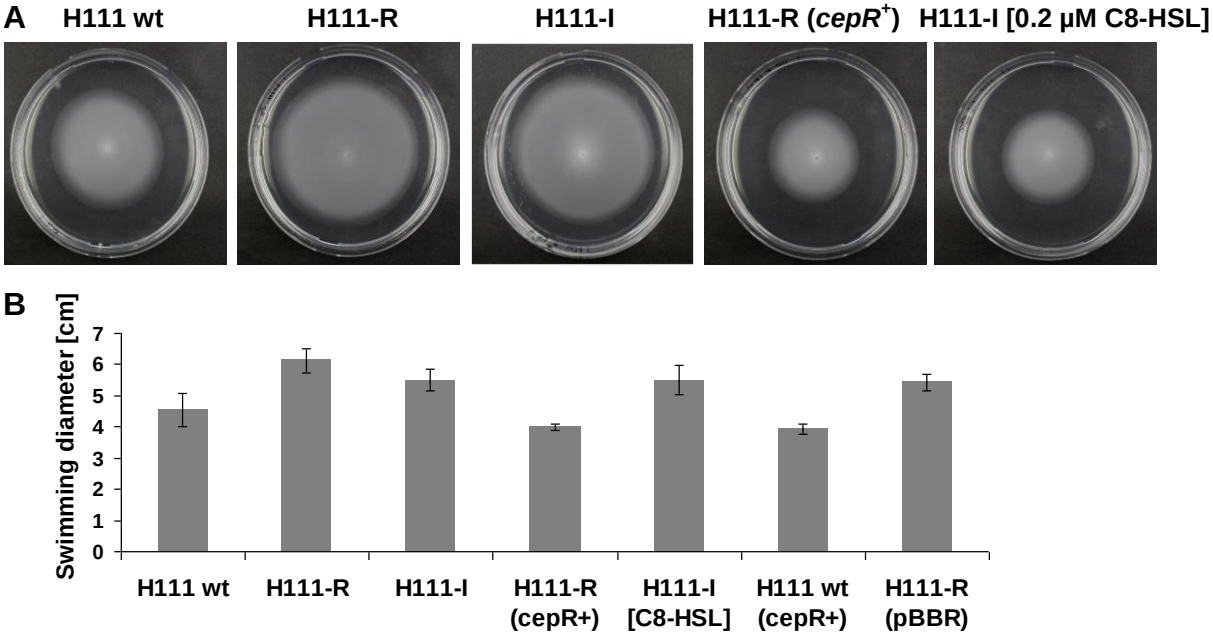


Fig. 7. Swimming activity of *B. cenocepacia* H111 wild type, mutants and complemented strains. (A) Bacterial cells of the wild type (wt), the *cepR* mutant H111-R, the *cepI* mutant H111-I and the *cepR* complemented strain H111-R (*cepR*$^+$) were spotted on AB minimal medium solidified with 0.2% agar and supplemented with 10 mM sodium citrate and 0.1% casamino acids (2.1.2). Swimming motility of the H111-I mutant was also examined in the presence of 0.2 µM *N*-octanoyl-homoserine lactone (C8-HSL). (**B**) Quantification of swimming motility was analysed after 48h of incubation at 30°C by measurement of swimming zones (2.4.5). Values are ± the standard error of the mean of $n = 4$.

The differences in swimming motility were most evident when the behaviour of the wild type was compared with the one of mutant H111-R (strain H111-R exhibited about 20% increased swimming, Fig. 7). An impact of QS regulation on the expression of flagellar genes has also been observed in *B. cenocepacia* strain K56-2 (O´Grady *et al*., 2009).

3.1.6 QS appears to only slightly affect *B. cenocepacia* H111 metabolism

The majority of the identified QS-regulated genes are involved in bacterial motility, adhesion, iron acquisition or the production of known or potential virulence factors (Table 5 and Table A1, Appendix). We wondered if the bacteria primarily utilise QS to trigger the expression of pathogenic traits or if cell-to-cell communication also affects primary metabolism of *B. cenocepacia* H111. To analyse the importance of QS for the utilization of nutrients we performed a metabolic profiling of the H111 wild type strain, mutant H111-R, and the complemented mutant H111-R (*cepR*$^{+}$) employing BIOLOG phenotypic microarrays (Fig. A2, Appendix, 2.4.7). The utilization of various carbon sources (PM1 and PM2), nitrogen (PM 3) and phosphorus and sulfur sources (PM 4), only showed minor differences: the mutant utilized L-threonine, D-arabinose, sorbic acid, D-glucosamine, thymine, thymidine, uracil, methylene diphosphonic acid and p-amino-benzene sulfonic acid better than the wild type (Table 6 and Fig. A2, Appendix). The most dramatic example was sorbic acid carbon source, since it could only be utilized by mutant H111-R but not the wild type. It is important to note that we did not detect any growth differences between the H111 wild type, mutant H111-R and the complemented *cepR* mutant when the strains were grown in LB-medium for the transcriptomic and proteomic investigations.

To validate the results obtained from the metabolic profiling the designated strains were also grown in liquid cultures or on agar plates (48h, 37°C) consisting of minimal medium (M9 minimal medium, (Johnson *et al*., 2008) or mineral salt medium, (Lindhardt TJ, 1989) (2.1.2), which was amended with selected C-sources including amongst others 10 mM citrate, butanol, catechol, L-threonine, L-histidine and D-arabinose. However, and in contrast with the results obtained with the BIOLOG plates, growth differences were only observed when the strains were grown in mineral salt medium with D-arabinose as C-source (Fig. A3, Appendix). It should be noted that these observations were made with strains grown on agar plates and it might be speculated that not only the bacterial growth but the production of extracellular polymeric substances (EPS) is affected by the presence of D-arabinose under these conditions (Fig. A3). Taken together, these results imply that QS does only marginally affect primary metabolism of *B. cenocepacia* H111. The observation that QS seems to have a

negative impact on bacterial growth might have been caused by the metabolic burden when QS induced concertedly many cellular functions.

Microarray	Substrate	Growth		
		H111 wt	H111-R	H111-R (*cepR*$^+$)
PM1 (Carbon)	L-Threonine	+	+++	+
PM2 (Carbon)	D-Arabinose	+	+++	-
	Sorbic acid	-	+	-
PM3 (Nitrogen)	D-Glucosamine	+	+++	+
	Thymine	++	+++	++
	Thymidine	++	+++	++
	Uracil	++	+++	++
PM4 (Phosphorus and Sulfur)	Methylene	-	++	+
	P-Amino-benzene sulfonic acid	-	+++	+++

Table 6. Growth differences between the H111 wild type (wt), the *cepR* mutant H111-R and the complemented mutant H111-R (*cepR*$^+$). BIOLOG phenotypical microtitre plates comprising different substrate components (2.4.7) were inoculated with bacterial suspensions in minimal medium. Plates were incubated at 37°C for 24h and bacterial growth was visually detected upon a blue colour development of the solution (Fig. A2, Appendix).

3.2 Project II: The role of the orphan LuxR homologue CepR2 in the transcriptional regulation of H111 genes and its effect on the quorum sensing circuitry

Previous work has identified the *B. cenocepacia* CepR homologous protein CepR2, which due to the absence of a chromosomal-encoded proximal LuxI-like protein is a member of the so-called orphan LuxR transcriptional regulators (A. Steidle, diploma thesis, TU Munich, 1999). CepR2 consists of 237 amino acids with a predicted molecular mass of 26,046 Da. The protein shares about 38% amino acid sequence identity with the *Ralstonia solanacearum* SolR protein (accession no. AAC45947) and the *Vibrio fischeri* LuxR regulator (AAQ90231), and 36% sequence identity with the *B. cenocepacia* CepR transcriptional regulator (AAD12726). As visualized by the alignment of deduced amino acid sequences (Fig. 8), CepR2 possesses all features which are characteristic for LuxR-response regulators: a helix-turn-helix DNA binding motif, the seven among LuxR proteins conserved amino acid residues, and a postulated AHL-binding domain (Fuqua *et al.*, 1994, Malott *et al.*, 2009).

```
CepR2           ---MDLTILHDCFDALQRAPTAEAAFPPIAAAAAALGFRYCVYGLRRTLPLARPDMQIVG
CepR            ----MELRWQDAYQQFSAAEDEQQLFQRIAAYSKRLGFEYCCYGIRVPLPVSKPAVAIFD
SolR            ----MGPGFQDAYHAFHTAQDERQLFRQIASVVRQLGFDYCCYGIRVPLPVSKPAVAIFD
LuxR            MNIKNINANEKIIDKIKTCNNNKDINQCLSEIAKIIHCEYYLFAIIYPHSIIKPDVSIID
                         ..  . :  .   .     ::     :   *  :.:  . .: :* : *..

CepR2           NHPREWEHRYVKFGYVTIDPIIKRVASQPRPVVWNAFDEP----GDTAFWHDAACFGMRY
CepR            TYPDSWMAHYQAQNYIEIDSTVRDGALSTNMIVWPDVDRI----DPCPLWQDARDFGLSV
SolR            TYPAGWMEHYQASGFLEIDPTVRTGASSSDLIIWPVSIRD----EAARLWSDARDFGLNI
LuxR            NYPEKWRKYYDDAGLLEYDPVVDYSKSHHSPINWNVFEKKTIKKESPNVIKEAQESGLIT
                .:*  *   *   . :  *. :         : *    .         .  :*   *:

CepR2           GWSHGGYDRAGNLGVLTLVRDTTPLDADEISRLRAPCASLAHAAHAYLMPRLADPIAP-V
CepR            GVAQSSWAARGAFGLLSIARHADRLTPAEINMLTLQTNWLANLSHSLMSRFMVPKLSPAA
SolR            GVARSSWTAHGAFGLLTLARRADPLTAAELEQLSATTNWLANLAHALMSPFLMPKLVPES
LuxR            GFSFPIHTASNGFGMLSFAHSDKDIYTDSLFLHASTNVPLMLPSLVDNYQKIN-TTRKKS
                * :       . :*:*::.:    : . .:         *   :       :

CepR2           GTGLTLREREVLAWTADGKTAYEIGMIFGIAERTVKFHLQNAVVKLDAMNKTHAATKAAM
CepR            GVTLTARELEVLCWTAEGKTACEIGQILSISERTVNFHVNNILEKLGATNKVQAVVKAIS
SolR            SAALTARELEVLCWTGEGKTAYEIGQILRISERTVNFHVNNVLLKLAATNKVQAVVKAIA
LuxR            DSILTKREKECLAWASEGKSTWDISKILGCSERTVTFHLTNTQMKLNTTNRCQSISKAIL
                .  ** **:* *.*:.:**:: :*. *:  :****.**: *   ** : *: ::  **

CepR2           LGLLP------
CepR            AGLIEAP----
SolR            IGLI-------
LuxR            TGAINCPYLKN
                 * :
```

Fig. 8. Amino acid sequence alignment of CepR2 with LuxR-type transcriptional regulators. Aligned are deduced amino acid sequences of the *B. cenocepacia* H111 CepR2 protein with the H111 CepR transcriptional regulator, *Ralstonia solanacearum* SolR and LuxR of *Vibrio fischeri* with * (identical amino acids), **:** (conserved amino acids), **.** (weakly conserved amino acids). Amino acids which are conserved among LuxR family proteins are marked by grey backgrounds. Regions that constitute the postulated AHL-binding domain are indicated by a bold line, and amino acids comprising the helix-turn-helix DNA binding motif are surrounded by a box (Fuqua *et al.*, 1994, Malott *et al.*, 2009).

3.2.1 Construction and characterization of a *cepR2* mutant in H111

The impact of CepR2 on *B. cenocepacia* gene expression was examined by the functional characterization of a H111 *cepR2* insertion mutant. The mutant strain, referred to as H111-R2, was constructed via single cross-over recombination events, and the interruption of *cepR2* was analysed by southern blot hybridization (Fig. 9A, 2.2.2.2). Complementation studies with CepR2 were achieved employing plasmid pJTR2, which encodes the H111 *cepR2* gene on plasmid backbone pBBR1MCS (2.2.5). To exclude an effect of CepR on the functioning of CepR2 when providing *cepR2* in *trans*, we also inactivated the *cepR2* gene in the *cepR* mutant H111-R, generating the double mutant H111-R/R2 (Fig. 9B). Several experiments were conducted to phenotypically characterize mutant H111-R2, including investigations on the production of extracellular proteases, swarming motility, and biofilm formation. Moreover, we examined the degree of antibiotic resistances in mutant H111-R2, the production of AHL signal molecules, its pathogenic potential in the *C. elegans* infection model, and the production of siderophores (see J. Toller, Master thesis, University of Zurich, 2008). The results obtained in these analyses strongly suggest that CepR2 functions as a regulator of pyochelin production.

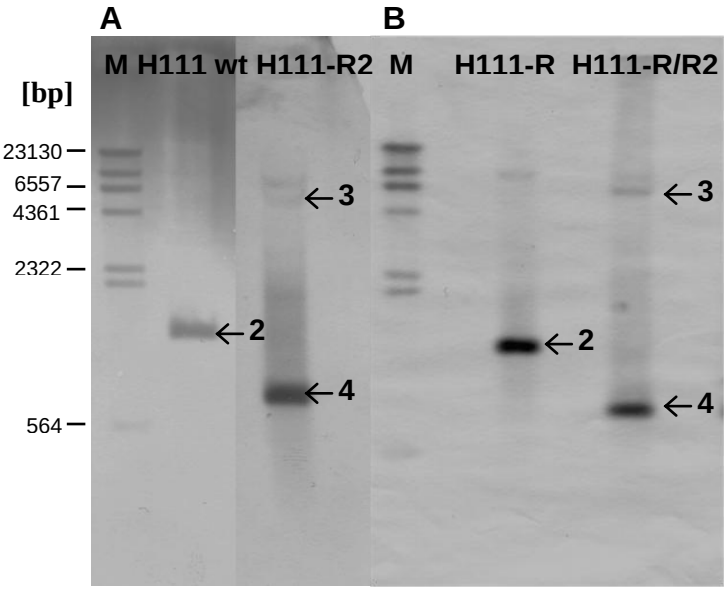


Fig. 9. Interruption of the *B. cenocepacia cepR2* gene via single cross-over recombination events. (**A**) CepR2 was mutated in the H111 wild type generating mutant H111-R2 and (**B**) in the *cepR* mutant generating mutant H111-R/R2. Mutations were generated by employing the replacement vector pEX18Gm-*cepR2* (2.1.1). Southern blot analyis (2.2.2.1) showed the hybridization of a DIG-labelled polynucleotide sonde to *Bam*HI-digested chromosomal DNA of the wild type and mutants, precisely to the unmodified *cepR2* encoding DNA fragment (**2**, 1287 bp) and to the plasmid interrupted *Bam*HI-digested *cepR2* fragments (**3**, 6808 bp and **4**, 688 bp).

3.2.2 CepR2 is involved in the production of pyochelin in *B. cenocepacia* H111

Burkholderia species produce up to four different siderophores: salicyl acid, cepabactin, ornibactin and pyochelin (Sokol, 1986), of which pyochelin and ornibactin are detectable on CAS plates (Schwyn, 1987). Whereas mutant H111-R2 was indistinguishable from the parent strain in most of the tested phenotypes, we discovered that the production of siderophores, precisely pyochelin, was greatly influenced by CepR2. When the *B. cenocepacia* strains were tested on CAS plates, no significant siderophore activity was observed with mutants H111-R2, the *cepR* mutant H111-R, and the double mutant H111-R/R2 (Fig. 10). The presence of *cepR2* on plasmid pJTR2 by contrast greatly stimulated the synthesis of iron-chelators to a degree that was well above the level of production observed in the parent strain. When *cepR* was provided in *trans* on plasmid pBBR-*cepR* (2.2.1), the mutant phenotype of H111-R, but not the one of H111-R2 or H111-R/R2, was rescued. These data indicate that the production of at least one siderophore is CepR2-regulated and suggest a regulatory cascade in which CepR is required for the expression of CepR2 in *B. cenocepacia* H111.

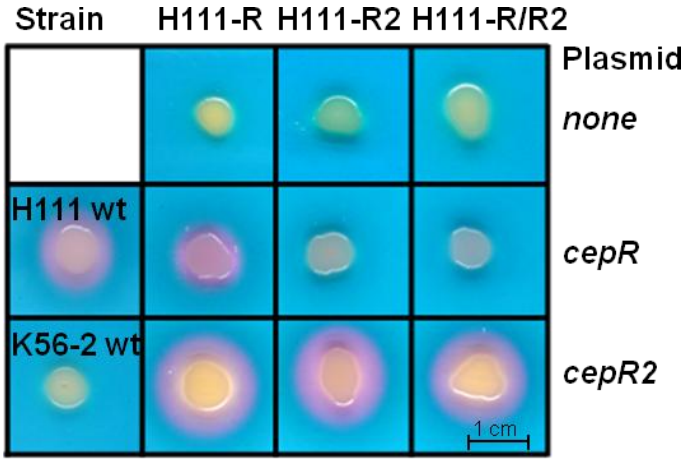


Fig. 10. CAS activity of *B. cenocepacia* strains. Siderophore activity of the H111 wild type (wt), the *cepR* mutant H111-R, the *cepR2* mutant H111-R2, and the *cepR cepR2* double mutant H111-R/R2, as well as activities of *cepR* or *cepR2* complemented mutants were analyszed on CAS plates. Plates were incubated for 2 days at 37°C. *B. cenocepacia* strain K56-2 was included as a control and is unable to produce pyochelin as a result of a point mutation in the pyochelin synthetase gene *pchF* (BCAM2228), (Holden *et al*., 2009).

To determine the contribution of pyochelin and ornibactin to the overall siderophore activity seen on CAS plates, supernatants of cultures grown in succinate medium were extracted with dichloromethane (2.4.4), and the organic and aqueous fractions were spotted on CAS plates (Fig. 11). Analyses of the organic fractions revealed that H111 produced only minute amounts of pyochelin under these conditions (Fig. 11A). Likewise, extracts of H111-R, H111-R2, or H111-R/R2 cultures showed no activity, and complementation of the mutant strains with *cepR* did not stimulate pyochelin production. Complementation with *cepR2* resulted in strong overproduction of pyochelin in all three mutants, indicating that CepR2 is a major regulator of pyochelin biosynthesis. Analysis of the aqueous fractions showed that CepR2 has no influence on ornibactin production (Fig. 11B). However, we observed that inactivation of

cepR stimulated ornibactin synthesis and that complementation of the mutants with *cepR* repressed ornibactin production. These results are in full agreement with previous work demonstrating that the CepIR system is a negative regulator of ornibactin biosynthesis in *B. cenocepacia* K56-2 (Lewenza & Sokol, 2001).

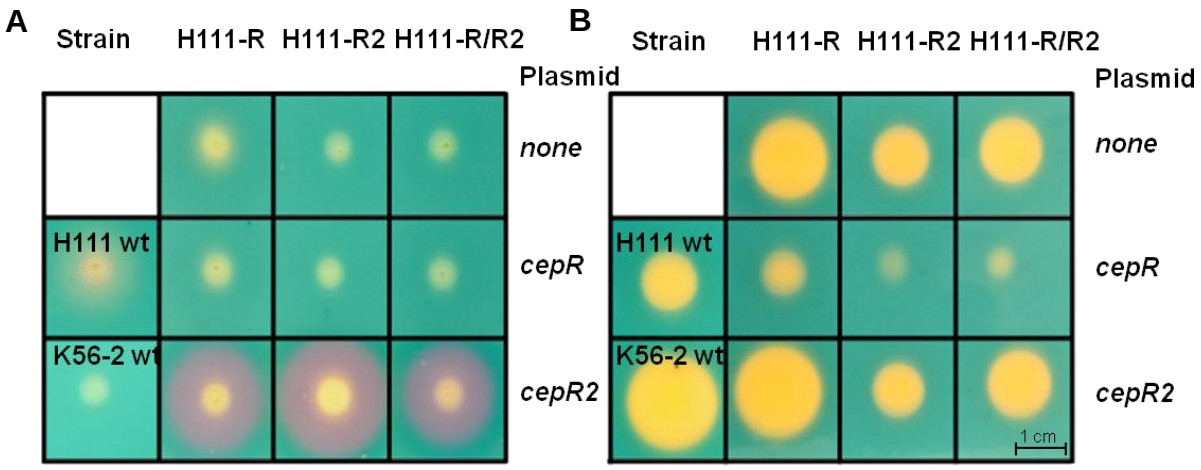


Fig. 11. Production of pyochelin (A) and ornibactin (B) in *B. cenocepacia* strains. Siderophore production is shown for the H111 parent (wt), the *cepR* mutant H111-R, the *cepR2* mutant H111-R2, and the *cepR cepR2* double mutant H111-R/R2, as well as for *cepR* or *cepR2* complemented strains. Supernatants of cultures grown in minimal medium were extracted with dichloromethane, and the organic and aqueous fractions were analysed separately on CAS plates (2.4.4). Extracts of supernatants of *B. cenocepacia* H111 and *B. cenocepacia* K56-2 were included as controls.

To further analyse the role of CepR2 in the regulation of pyochelin biosynthesis, the promotorless *lacZ* gene was fused to the promoter regions of *pchR* (BCAM2231, encoding an AraC family transcriptional regulator of pyochelin production) and *pchD* (BCAM2232, the first gene in the pyochelin biosynthesis gene cluster, 2.2.8). In LB-medium no β-galactosidase activity was detected examining the *pchD* promoter construct (Fig. 12B) while fusion P_{pchR}-*lacZ* showed high activity in the early growth phase in both H111 wild type and H111-R (Fig. 12A). Consistent with our data on pyochelin production, the P_{pchR}-*lacZ* fusion did not show any activity in H111-R2. In succinate minimal medium both promoters were active in the H111 parent (Fig. 12C and D). As it was in LB-medium, expression of the P_{pchR}-*lacZ* fusion was dramatically decreased in the *cepR2* mutant, while the activity of fusion P_{pchD}-*lacZ* was slightly reduced in mutant H111-R2. These data support a model in which CepR negatively regulates expression of ornibactin biosynthesis genes and positively regulates expression of *cepR2*, which in turn is required for full expression of pyochelin biosynthesis genes.

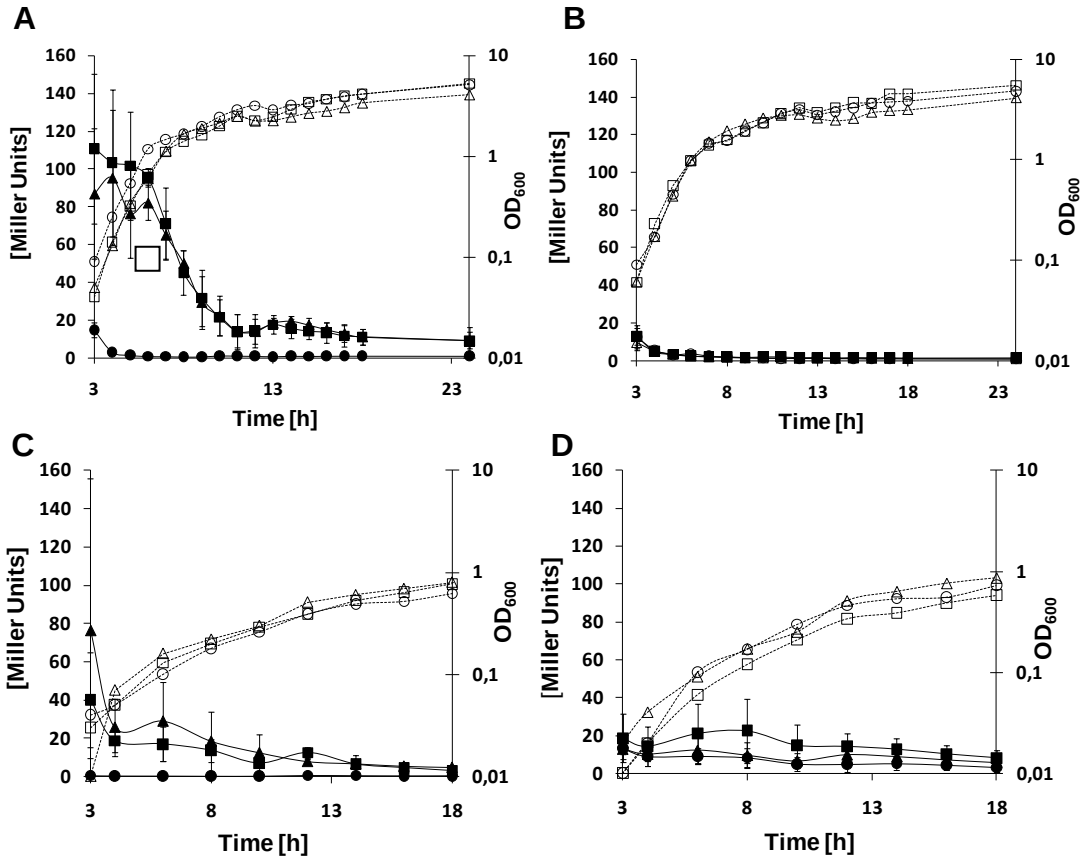


Fig. 12. CepR2 controls the activity of the *pchR* promoter. (**A** and **C**) Activities of the *pchR* (BCAM2231) promoter driving expression of the pyochelin gene cluster (BCAM2231 to BCAM2221) and (**B** and **D**) of the *pchD* promoter (BAM2232) driving expression of the pyochelin biosynthesis operon (BCAM2232 to BCAM2235) were determined using LB-medium (**A** and **B**) and succinate medium (**C** and **D**). Growth (open symbols) and β-galactosidase activities (filled symbols) were monitored throughout the growth curve as measured by absorbance at 600 nm (OD_{600}). The transcriptional fusions were analysed in the H111 parent (squares), the *cepR* mutant H111-R (triangles), and in the *cepR2* mutant H111-R2 (circles). Values are means ± standard deviations (n = 3).

3.2.3 CepR2 – an AHL-independent transcriptional regulator

While examining siderophore activities in strain H111, we observed that the production of pyochelin by the *cepR* mutant and the *cepR cepR2* double mutant was restored by complementation with *cepR2* (Fig. 10 and Fig. 11A). As neither of these mutants produces AHL molecules, we hypothesized that CepR2 may not require signal molecules for its activity. To test this hypothesis, plasmid pJTR2 (2.2.1) was transferred into *E. coli* MT102 harbouring the green fluorescent protein *gfp*-based AHL sensor plasmid pJBA89 (Fig. 13A and 2.4.2.1). To rule out the possibility that the *luxR* gene encoded on pJBA89 influences the activity of CepR2, *luxR* was moreover deleted generating plasmid pJBA89*luxR*$^-$ (Fig. 13B - D and 2.4.2.3). The reporter activity of all strains was determined in LB-medium (2.4.2.3).

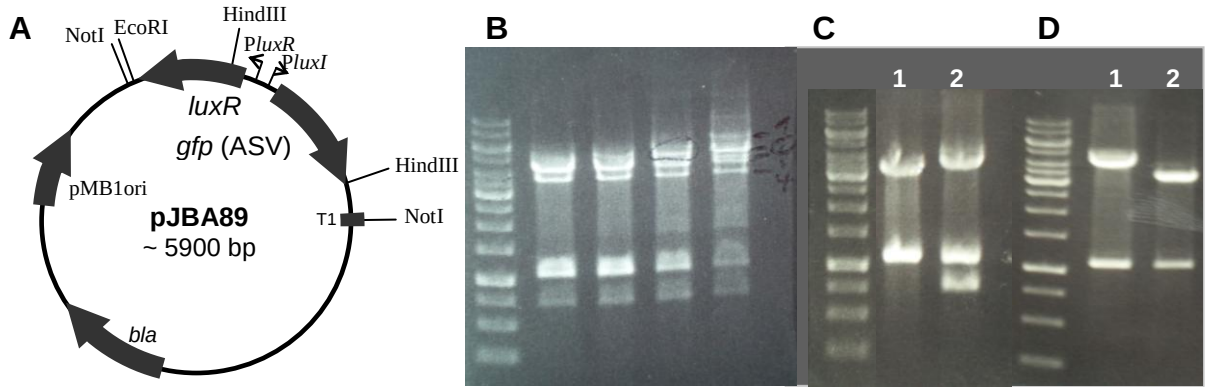


Fig. 13. Deletion of *luxR* from plasmid pJBA89 by partial digestion with *Hin*dIII. (**A**) DNA was extracted from plasmid pJBA89, plasmid map after (Andersen *et al.*, 2001, Winson *et al*., 1998). (**B**) The *Eco*RI-linearized DNA was partially digested with restriction enzyme *Hin*dIII, then separated by agarose gel electrophoreses for the preparative analyses. Generation of plasmid pJBA89*luxR*$^-$ (2.2.6, deletion of a 703 bp-fragment containing *luxR*) was verified by digestion with restriction enzymes *Eco*RI and *Hin*dIII (**C**) and *Hin*dIII (**D**) of plasmid DNA extracted from pJBA89 (**1**) and pJBA89*luxR*$^-$ (**2**).

As shown in Fig. 14A, plasmid pJBA89 is based on the genetic elements of the LuxIR QS system of *Vibrio fischeri* and thus is most sensitive to 3-oxo-C6-HSL, but it is also responsive (with decreased sensitivity) to various related AHL molecules. In the presence of *cepR2* (pJTR2, 2.2.1), the GFP expression of the sensor plasmid was strongly induced, and addition of AHL signal molecules did not further increase the fluorescence (Fig. 14C). Plasmid pJBA89*luxR*$^-$ could, as expected, not be activated by the external addition of AHL molecules (Fig. 14B). However, when plasmid pJTR2 was provided in *trans*, GFP expression was strongly stimulated independent of the presence or absence of AHL molecules (Fig. 14D). Taken together, these results strongly suggest that CepR2 acts as a transcriptional activator in an AHL-independent manner.

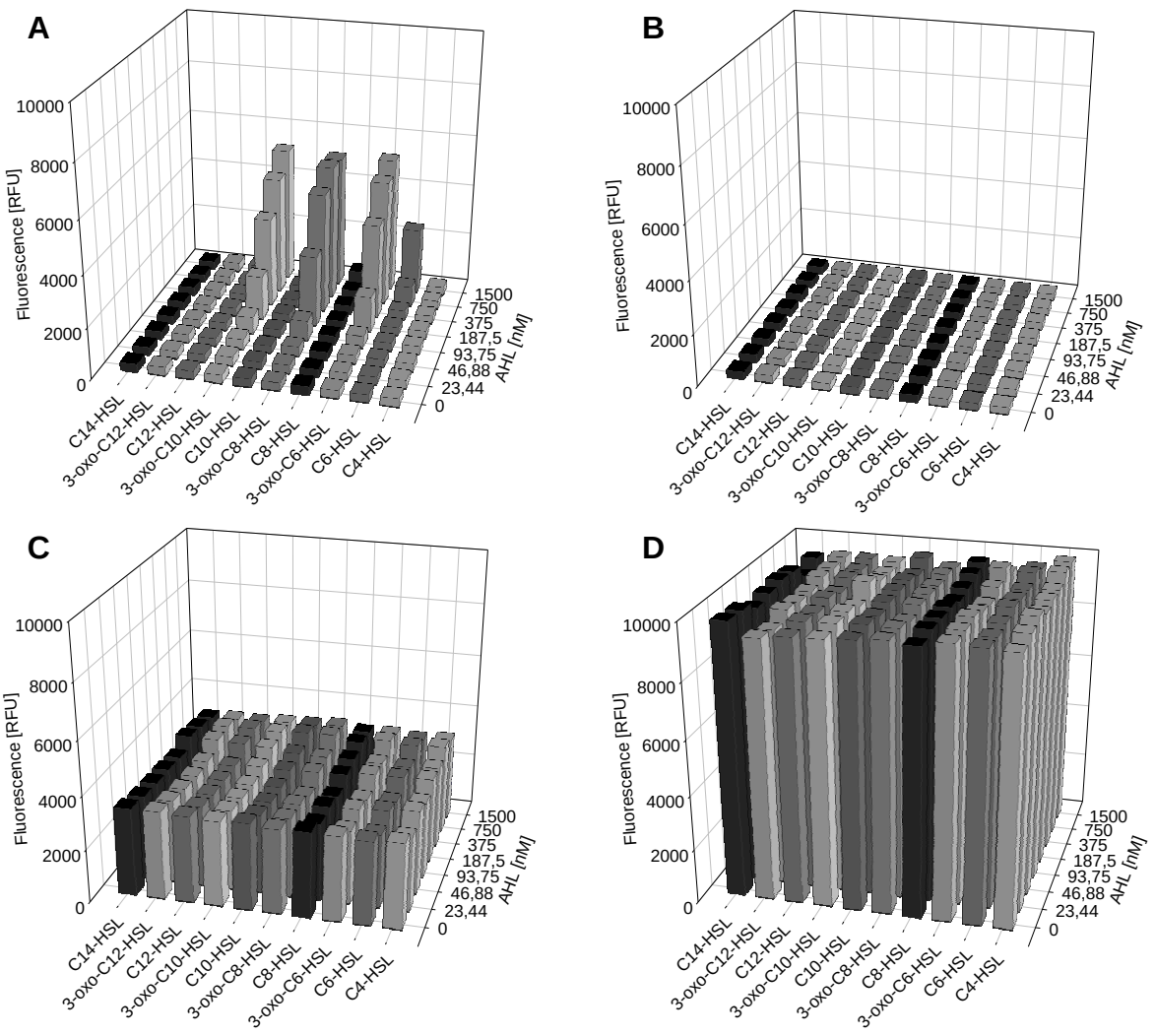


Fig. 14. CepR2 activates a P_{luxI}-*gfp* transcriptional fusion in an AHL-independent manner. The GFP expression (RFU, relative fluorescence units) of the AHL biosensor *E. coli* MT102 (pJBA89) (**A** and **C**) and its *luxR* deletion derivative *E. coli* MT102 (pJBA89*luxR*$^-$) (**B** and **D**) was measured in the absence (**A** and **B**) and in the presence (**C** and **D**) of plasmid pJTR2. Measurements were also obtained in the absence or presence of various amounts of different AHL signal molecules (2.4.2.3), (Malott *et al*., 2009).

As the sensor plasmid contains a *lux* box operator sequence that is similar but not identical to the *cep* box sequence of *B. cenocepacia* (Lewenza *et al*., 1999) we also analysed the effect of CepR2 on the transcription of the H111 *cepI* gene. For this purpose we determined the activities of the promoter fusion P_{cepI}-*lacZ* (2.2.8) in an *E. coli* background while simultaneously providing *cepR2* on plasmid pAH1B.1 (2.2.1). The activities were very low (1 Miller Unit) independent of whether *cepR2* was present in *trans*, moreover, the addition of 200 nM C8-HSL to the medium did not affect transcriptional activities (data not shown). These observations indicate that CepR2 does not recognize the *cep* box sequence upstream of *cepI* but may bind a somewhat different operator sequence that is more similar to a *lux* box sequence. Since we observed that the transcription of the pyochelin regulator *pchR* is

dependent on CepR2 (Fig. 12), we also measured the activity of promoter fusion P_{pchR}-*lacZ* in *E. coli* in the presence or absence of *cepR2* as before. Here, we observed an approximately threefold C8-HSL-independent induction of β-galactosidase activities when *cepR2* was present, however, promoter activities were very low (1.3 ± 0.6 and 5.2 ± 1.9 Miller units in the absence and presence of *cepR2*, respectively). Given the strong effect of CepR2 on the transcription of *pchR* in H111, it might be speculated that either additional host factors are required for full activation of the promoter, or that CepR2 controls the expression of a downstream regulator, which stimulates *pchR* transcription. The fact that the inspection of putative *pchR* promoter regions did not reveal any obvious *lux* or *cep* box-like sequences may favour the latter possibility.

3.3 Project III: Characterization of the BclACB lectins of *B. cenocepacia* H111, examination of their expression, subcellular localization and their role in biofilm formation

The following section describes the characterization of the *B. cenocepacia* H111 lectin-deficient mutant H111-*bclACB* including the impact of the BclACB proteins on the structural development of biofilms. Moreover, in this chapter data are presented demonstrating that transcription of the *bclACB* operon is QS-regulated. It is also shown, that expression of lectin BclB is growth phase-dependent and the subcellular localization of lectin BclB by immunofluorescence labelling experiments is investigated.

3.3.1 BclACB lectins are required for biofilm structural development

To understand the impact of the BclACB lectins on cellular functions in *B. cenocepacia* H111, a lectin-deficient mutant, named H111-*bclACB*, was constructed. This mutant was generated by allelic replacement of the H111 lectin-encoding operon *bclACB* (BCAM0186 - BCAM0184, Fig. 15 and Fig. 20) using a modified protocol of the Gateway cloning technology (2.2.4.2), (Carlier *et al*., 2009). Allelic replacement was achieved employing plasmid pAUC40-bcl, which contains a kanamycin cassette embraced by flanking regions of the *bclACB* genes (Fig. 15A). Positive clones were analysed by PCR as illustrated in Fig. 15B and the deletion of the lectin-encoding genes was additionally verified by Western immunoblot analyses (see 3.3.4).

The Bcl proteins are homologues of the *Pseudomonas aeruginosa* fucose-binding lectin PA-IIL, also referred to as LecB (Lameignere *et al*. 2008). PA-IIL-family proteins are thought to play important roles in processes that require cell adhesion, host recognition or defence mechanisms (Tielker *et al*., 2001, Sudakevitz *et al.*, 2002, Zinger-Yosovich *et al*., 2006, Imberty *et al.*, 2005, Imberty and Varrot 2008). For the characterization of mutant H111-*bclACB* we therefore focused on phenotypes that most probably require the attachment of the bacteria. These phenotypes included the ability to swim and swarm (2.4.5), the formation of biofilms under both static and dynamic conditions (2.4.6) and the potential to cause infection in the nematode *C. elegans* or the greater wax moth *Galleria mellonella* (2.4.8). As described in the following paragraph, mutant H111-*bclACB* exhibited differences when compared to the wild type in the structural development of biofilms in flow-through cells and in regard to its pathogenic potential in the nematode *C. elegans* (see 3.3.2). However, the deletion of *bclACB* did neither affect the ability of the bacteria to swim or swarm, nor to cause disease in *G. mellonella* (data not shown).

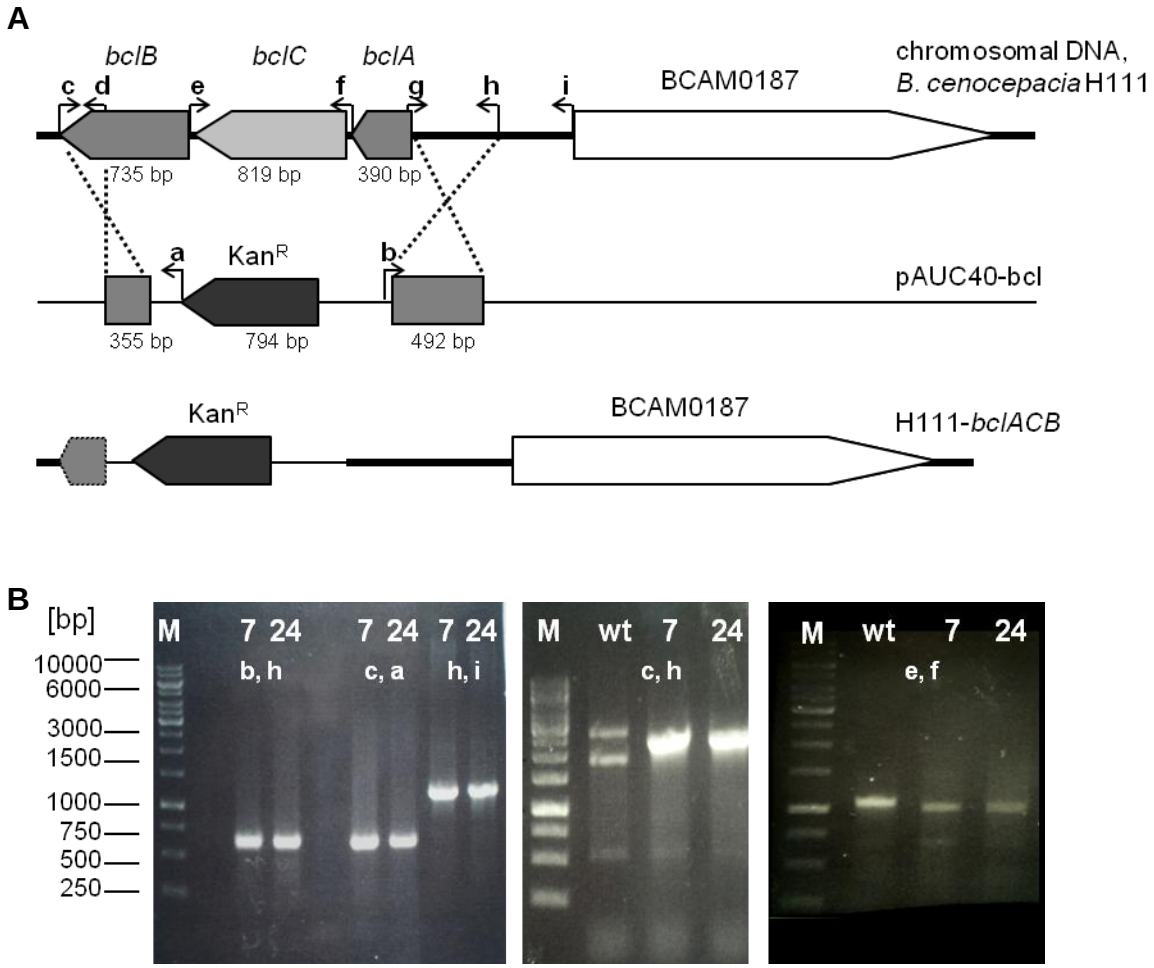


Fig. 15. Generation of the lectin deletion mutant H111-*bclACB*. The *bclACB* genes were deleted using a gene replacement procedure as shown. (**A)** Primers used are (2.1.3): kanDnFwd (a), kanUpRev (b), lecDn-GW (c), lecDn-kan (d), lecB2-up (e), lecB2-dn (f) lecUp-kan (g), lecUp-GW (h) and lecB4P (i) with M (DNA marker, 2.2.2). (**B**) Deletion of *bclACB* was verified by PCR employing primers used in A and chromosomal DNA of the H111 wild type (wt) and H111-*bclACB* mutant clones 7 (7) and 24 (24).

We were unable to detect significant differences in biofilm formation between the H111 wild type and mutant H111-*bclACB* when strains were inoculated from overnight cultures in polystyrene microtitre-dishes and grown for 48h at 30°C (Fig. 16A, 2.4.6). This observation was independent of whether the AB minimal medium used in this experiment was supplemented with 10 mM sodium citrate or 10 mM D-glucose. In contrast, the *cepR* mutant H111-R, which was included in these experiments as a control, was defective in biofilm formation under both conditions. In some assays we observed that the *bclACB* mutant was slightly impaired in its ability to develop biofilms when strains were incubated for shorter time periods at 30 °C (12h or 24h). These observations suggested that the Bcl proteins might affect the early stages of biofilm formation. To test this hypothesis, overnight cultures of bacteria were inoculated to an optical density (OD_{600}) of 0.2 into wells of microtitre-dishes,

which were then placed at 30°C for a period of 6h (Huber *et al*., 2002). Interestingly, under these conditions, the biofilm mass was decreased by about 20% in mutant H111-*bclACB* in comparison to the wild type (Fig. 16B). These differences were, however, only observed when strains were grown in AB minimal medium supplemented with 10 mM sodium citrate and not in medium amended with glucose. As described in section 3.3.3, transcription of the *bclACB* operon was found to be induced by the transcriptional regulator CepR2. For that reason we also included the *cepR2* mutant H111-R2 in these experiments. Notably, similar to the observation made with the H111 *bcl* mutant, mutation of *cepR2* did not affect the development of biofilms in microtitre-dishes when cells were adjusted from overnight cultures to an OD_{600} of 0.01 and incubated for a period of 48h (Malott *et al*., 2009). However, when cells were adjusted to an OD_{600} of 0.2 and grown for 6h at 30°C in AB medium supplemented with citrate, strain H111-R2 showed a reduction of approximately 30% in biofilm mass relative to the wild type, similar to what was observed with mutant H111-*bclACB* (Fig. 16B).

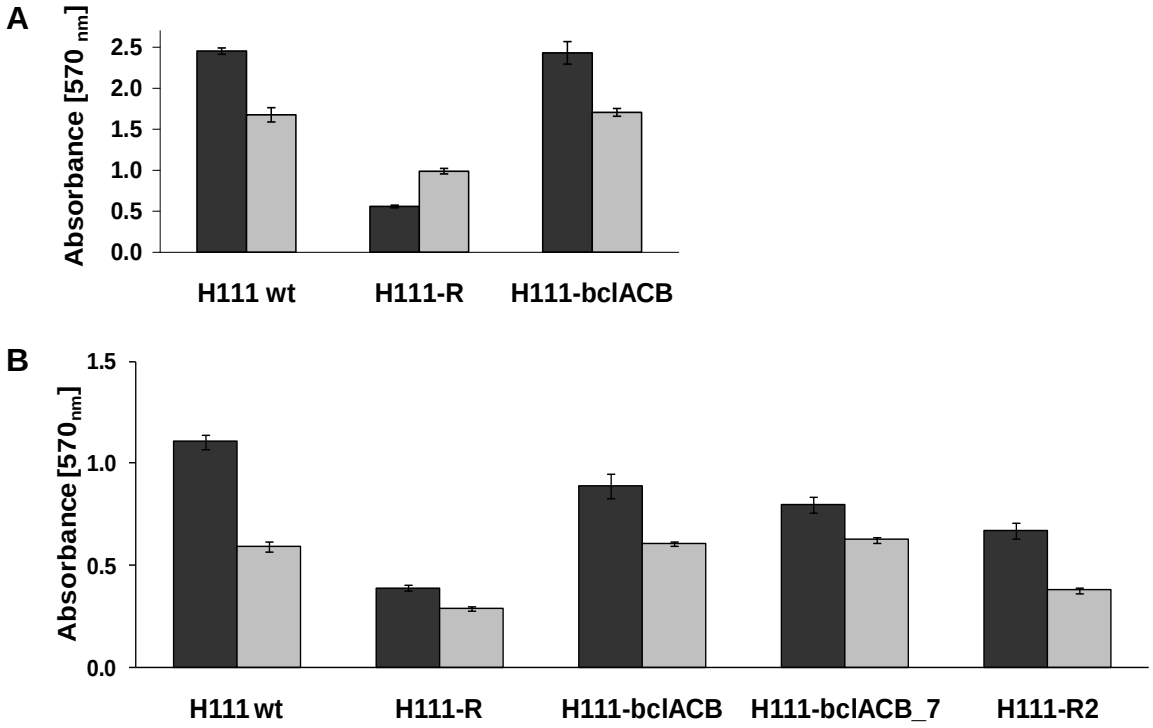


Fig. 16. Biofilm formation of *B. cenocepacia* H111 wild type and mutants in polystyrene microtitre-dishes. (**A**) *B. cenocepacia* wild type (wt), the *cepR* mutant H111-R, mutant H111-*bclACB*, H111-*bclACB* mutant clone 7 (H111-*bclACB*_7), and the *cepR2* mutant H111-R2 were grown in buffered AB minimal media (pH 7) supplemented with 10 mM sodium citrate (dark grey bars) or 10 mM D-glucose (light grey bars). Cells were adjusted from overnight cultures to an OD_{600} of 0.01 and incubated for 48h at 30°C in wells of polystyrene microtitre-dishes or, (**B)** for the analyses of initial attachment, to an OD_{600} of 0.2 followed by an incubation period of 6h at 30°C. After the incubation planktonic cells were removed and attached cells were stained with crystal violet (2.4.6.1). Error bars represent the standard error of the mean of six independent wells.

We also analysed the development of biofilms of mutant H111-*bclACB* in flow-through cells, which allows the visualization of biofilm development and the determination of biofilm structures (Christensen *et al*., 1999). For that purpose, the wild type and mutant strain were genetically tagged with the green fluorescent protein (GFP) and biofilm development was investigated over 96 hours. As depicted in Fig. 17A, cells of the wild type usually attach to the surface and proliferate to form characteristic irregularly shaped microcolonies, thereafter the void space between the microcolonies becomes colonized to cover the entire substratum eventually (Huber *et al*., 2002). As depicted in Fig. 17B, we observed significant differences in cell-attachment and biofilm structure that were unique for the lectin-deficient mutant H111-*bclACB*: the mutant was impaired in the initial colonization of the glas surface but then the cells aggregated into comparatively tall microcolonies, which were visible already 24h post inoculum (Fig. 4B). The colonies then developed into hollow volcano-like structures, in which the inner region remained uncolonized. These structures, which were most evident after 72h of biofilm development, then often converged at the upper part to form cupola-like structures whereas others remained open.

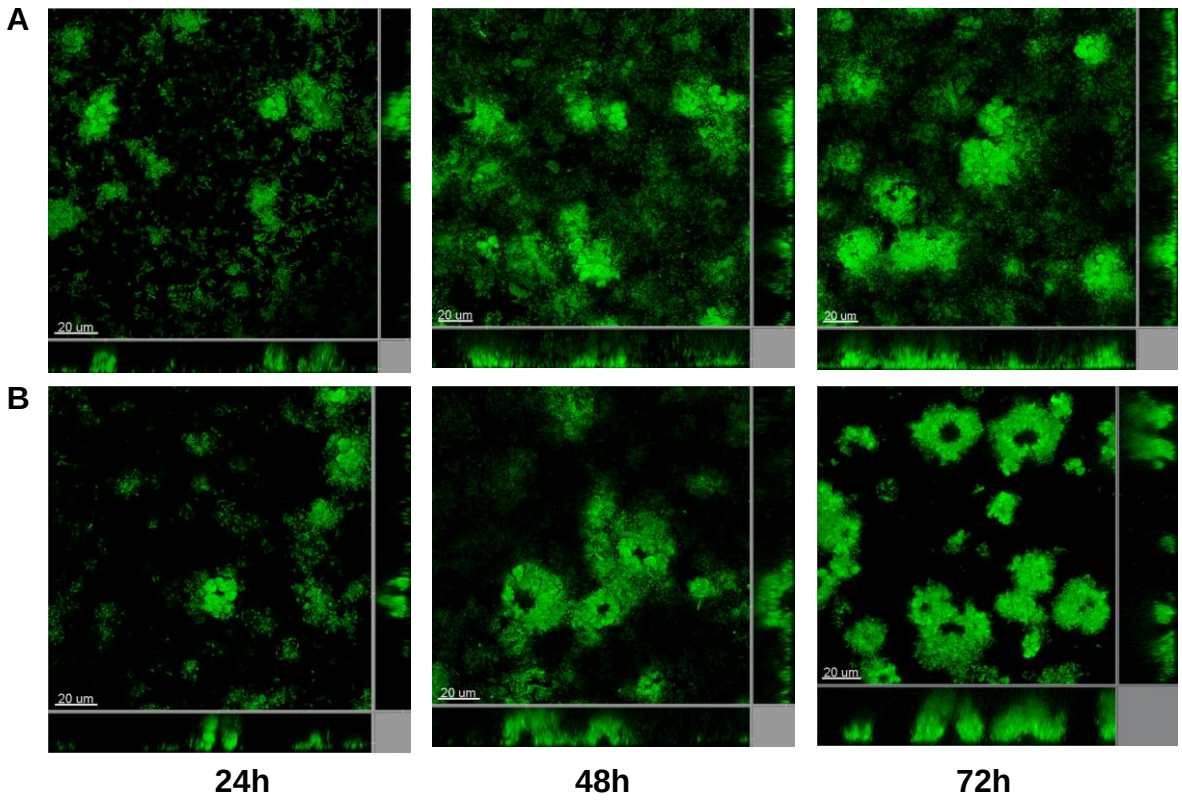


Fig. 17. Biofilm structures of the *B. cenocepacia* H111 wild type and mutant H111-*bclACB*. (**A**) Flow chambers were inoculated with *gfp*-tagged derivatives of the H111 wild type and (**B**) lectin mutant H111-*bclACB*. Biofilms were grown in buffered AB minimal medium (pH 7) supplemented with 10 mM D-glucose (2.4.6.2). CLSM pictures were taken at 24h, 48h and 72h post-inoculation.

Notably, in aged biofilms of the H111 wild type (incubation periods of 96h or later) we also observed the appearance of hollows but these were generally smaller in size. Staining with propidium iodide (2.4.6.3) moreover indicated that these hollows are the result of localized cell death (Fig. 18A), as it has been previously described for aged *P. aeruginosa* biofilms (Webb *et al.*, 2003), (Ma *et al.*, 2009). In contrast to an aged wild type biofilm the hollows observed in a H111-*bclACB* biofilm did not contain dead cells (Fig. 18B), suggesting that the formation of these structures is the result of an altered cellular aggregation behaviour of the strain rather than a consequence of cell death.

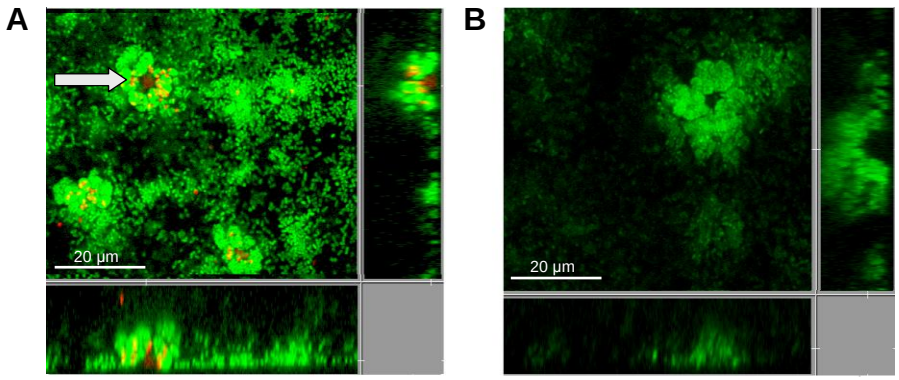


Fig. 18. Hollow colonies in mutant H111-*bclACB* are not due to cell death. (**A**) To visualize bacterial death within biofilms, *gfp*-tagged cells of the *B. cenocepacia* H111 parent and mutant H111-*bclACB* were stained with propidium iodide (2.4.6.3). The arrow indicates dead cells visible in the inner part of microcolonies formed by the H111 wild type at 96h post-inoculation. (**B**) Hollow microcolonies were observed already at 48h of biofilm development in the *bcl* mutant however no cell death was detected in the inner part of the microcolonies after staining with propidium iodide.

3.3.2 The Bcl orthologues are required for virulence in a *C. elegans* infection model

Many persistant and chronic infections are intrinsically linked to the ability of a pathogen to form biofilms (Davies, 2003). Moreover, lectins have been recognized as domains of bacterial toxins (Imberty *et al.*, 2005). For these reasons, we tested the pathogenicity of lectin mutant H111-*bclACB* using the nematode *Caenorhabditis elegans* and the larvae of the greater wax moth *Galleria mellonella* as infection hosts (2.4.8). When tested in the *C. elegans* infection model (Fig. 19), mutant H111-*bclACB* showed a modest but significant attenuation ($p < 0.05$; $n = 8$), indicating that the lectins may contribute to the strong nematode pathogenicity of strain H111 (Köthe *et al*., 2003), (Uehlinger *et al*., 2009). By contrast, no differences between the wild type strain and the lectin mutant were observed when determining the pathogenic potential in the *G. melonella* infection model (data not shown).

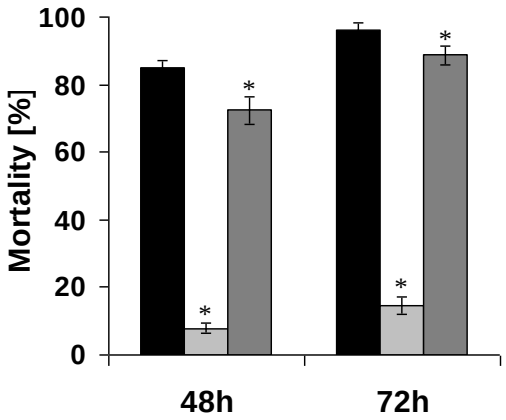


Fig. 19. Killing of nematodes is reduced in *B. cenocepacia* mutant H111-*bclACB* in comparison to the wild type. *Caenorhabditis elegans* slow-killing assays were performed with the H111 wild type (black bars), mutant H111-R (light-shaded bars) and mutant H111-*bclACB* (dark-shaded bars) after an incubation of 48h and 72h. Error bars represent the standard error of the mean of $n = 8$. The asterisk indicates significant differences when compared to the wild type (Student's *t*-test, $p < 0.05$).

3.3.3 Expression of the *bclACB* operon is CepR-induced and dependent on CepR2

Both the transcriptome and the proteome analyses revealed that the expression of the lectin-encoding operon, comprising genes *bclA* (BCAM0186), *bclC* (BCAM0185) and *bclB* (BCAM0184), is QS-regulated (see 3.1 and Fig. 20). Transcript levels of *bclA* were -9.0-fold reduced in the *cepR* mutant and transcript levels of *bclC* and *bclB* were -3.3-fold and -5.1-fold reduced in mutant H111-R. Moreover, protein levels of BclA and BclB were diminished in the whole cell fractions of the H111-R and H111-I proteomes (see 3.1 Fig. 6 and Table A3, Appendix), and amounts of BclA were decreased in the extracellular protein fractions of both mutant strains (Table A3). In support of our findings, H111 BclC has been previously identified as a *cep*-regulated homologue of PA-IIL family proteins (Riedel *et al*., 2003). Because of the observed similarity in the extent of QS regulation, and due to the fact that we did not identify promoter regions upstream of genes *bclC* or *bclB*, it seems most likely that the three lectins comprise a co-transcribed unit, which, in this study, is referred to as the *bclACB* operon.

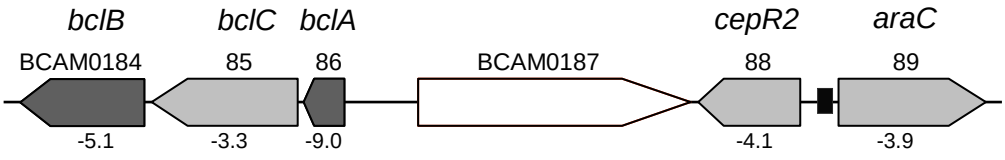


Fig. 20. Organisation of the *bclACB* genes on *B. cenocepacia* chromosome 2. The potential lectin operon *bclACB* comprises genes *bclA* (390 bp), *bclC* (819 bp) and *bclB* (735 bp). The orphan LuxR homologue CepR2 (BCAM0188, see 3.2) is encoded downstream of the *bclACB* operon. A potential *cep* box motif (CTGTTCAAAAGGACAGTT, black box) has been identified within potential promoter regions of the *araC* gene.

The sequence inspection of the putative promoter regions of *bclA* (we examined genome sequences of *B. cenocepacia* J2315 and H111) did not reveal the presence of any obvious *lux* or *cep* box like elements which are typically found within promoter sequences of *cep*-regulated genes, as for instance *cepI* or *aidA* (Huber *et al*., 2001, Huber *et al*., 2004, Fig. 6). The QS-dependent transcription of the *bclACB* genes was therefore confirmed by performing ß-galactosidase assays (2.4.1). Transcriptional activities of the *bclA* gene (promoter fusion P_{bclA}-*lacZ*, 2.2.8) were determined in the wild type, the *cepR* mutant H111-R and in backgrounds of the *cepR2* mutant H111-R2, as the proximal gene location of *cepR2* and *bclA* (Fig. 20) suggested an impact of CepR2 on *bclA* expression. As depicted in Fig. 21, mutations in *cepR* and *cepR2* similarly affected P_{bclA}-*lacZ* activities, leading to reduction of about half of the transcriptional activity that was determined in the wild type. These results strongly suggest that *bclA* transcription is dependent on the CepR protein and its homologue transcriptional regulator CepR2. The observations moreover favour our model in which CepR is required for the expression of CepR2, which in turn acts as a transcriptional regulator of gene expression via the binding to yet undefined operator sequences at promoter regions (3.2), (Malott *et al*., 2009). However, as P_{bclA}-*lacZ* activities are not completely lost in the absence of the downstream regulator CepR2, it seems likely that the transcription of *bclA* is regulated in conjunction with other yet unidentified genetic elements in *B. cenocepacia* H111.

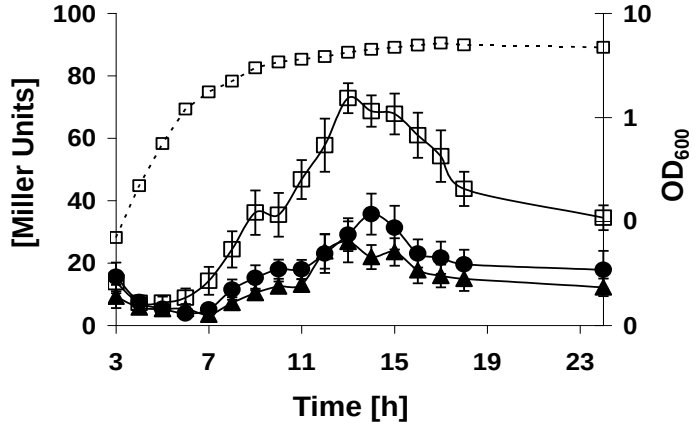


Fig. 21. Transcription of *bclA* is induced by CepR and CepR2. A transcriptional fusion of the *bclA* gene (P_{bclA}-*lacZ*) was assayed for ß-galactosidase activities in the *B. cenocepacia* H111 parent (open rectangles), the *cepR* mutant H111-R (closed triangles), and the *cepR2* mutant H111-R2 (closed circles) throughout the growth curve. Growth of the H111 wild type (dashed line) is reflected by measurement of absorbance at 600 nm (OD_{600}). Strains were grown in LB-medium and values are means ± standard error of the mean (n = 3).

3.3.4 Analyses of BclB expression by Western immunoblotting

The QS-dependent expression of the Bcl proteins was also revealed by Western immunoblot analysis using polyclonal antibodies raised against the purified BclB protein (BCAM0184 or BC2L-B, Fig. 20). As expected (Fig. 22A), amounts of BclB were absent in fractions of whole cell proteins extracted from lectin mutant H111-*bclACB* and the *cepR* mutant H111-R.

Protein levels could be restored when *cepR* was provided in *trans*. The microarray profiling (see 3.1) and the analysis of promoter activites of *bclA* suggested a positive impact of the transcriptional regulator CepR2 on the expression of BclACB. For that reason we also analysed the amounts of BclB in extracts of whole cell proteins belonging to mutant H111-R2. However, and in contrast to our expectations, BclB amounts were not diminished in the *cepR2* mutant strain in comparison to wild type (Fig. 22A). Interestingly, the cellular presence of BclB seemed to be restricted to a short period within the stationary-phase of growth, since BclB amounts were greatly reduced in the whole cell protein fractions of cultures that were grown overnight (OD_{600} >4) (Fig. 22A). Lectin BclB was also identified in the H111 extracellular protein fractions (Fig. 22B) which were extracted from corresponding culture supernatants that were used for the whole cell analyses (2.3.1). Here, BclB levels were also reduced in extracellular protein extracts belonging to cultures of late-stationary phases. For that reason it seems unlikely that the decrease in BclB amounts observed when analysing whole cell proteins of an OD_{600} >4 occurred because of a cell lysis.

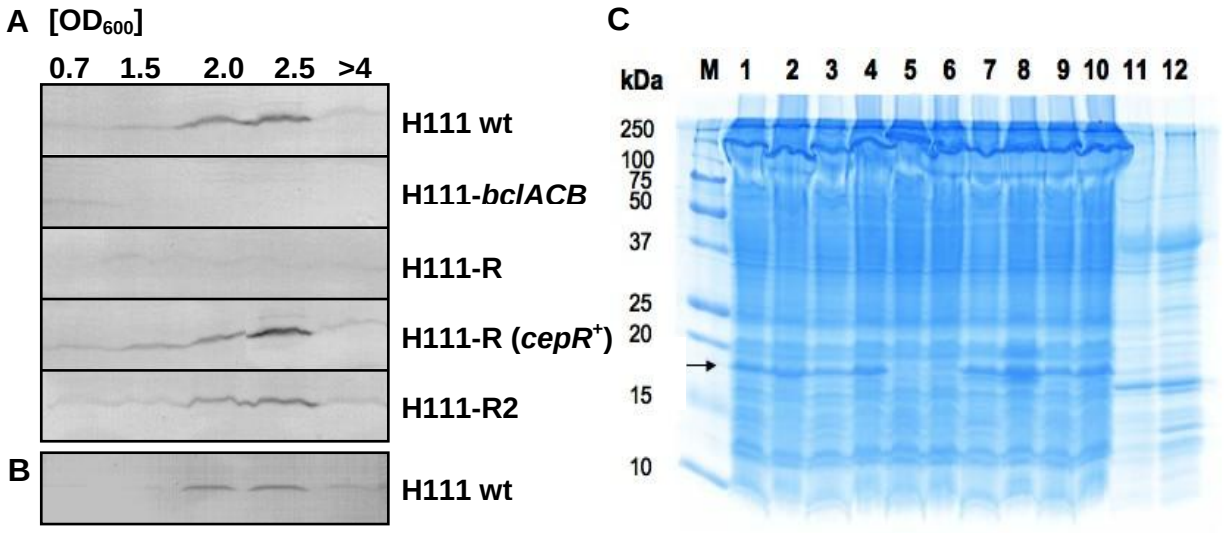


Fig. 22. QS-dependent expression of lectin BclB. Depicted is a Western immunoblot analyses of the *B. cenocepacia* H111 wild type (wt), lectin mutant H111-*bclACB*, the *cepR* mutant H111-R, the *cepR2* mutant H111-R2, and the *cepR* complemented strain H111-R (*cepR*$^+$) using polyclonal antibodies directed against the purified BclB protein. BclB was detected employing fractions of whole cell proteins (**A**) or extracellular proteins (**B**) extracted from culture supernatants of samples in A (2.3.1). Strains were grown in LB-medium and samples of 50 ml volumes were taken at different cellular densities over a period of 24h as determined by absorbance at 600 nm (OD_{600}). In all cases, equal amounts of proteins were loaded, as visualized by Coomassie-blue staining of proteins separated on a 15% SDS-PAGE (**C**) (2.3.2). Proteins loaded on the gel were extracted from cultures of OD_{600} 2.5 and >4 of whole cells belonging to the wild type (1,2), mutant H111-*bclACB* (3,4), mutant H111-R (5,6), the complemented mutant H111-R (*cepR*$^+$) (7,8) and mutant H111-R2 (9,10), and of H111 wild type extracellular protein fractions (11,12). The QS-dependent expression of AidA (indicated by an arrow) served as a control.

3.3.5 Lectin BclB is associated with the bacterial surface

Recent work has described the protein structures of *B. cenocepacia* lectins BclA (Lameignere *et al*., 2008, Lameignere *et al*., 2010) and BclC (Sulak *et al*., 2010), as well as their binding affinity to carbohydrate ligands. The subcellular localization of the Bcl orthologues has, however, not been studied in detail. The finding that the lectin-deficient strain H111-*bclACB* was impaired in the structural development of biofilms prompted us to determine the surface-associated localization of the Bcl proteins. For this purpose, we performed immunofluorescence labelling reactions where we used Alexa Fluor® 594-conjugated antibodies raised against the purified H111 BclB protein (see 2.3.5). Samples of the *B. cenocepacia* H111 wild type which were tagged with the *gfp*-expressing plasmid pBAH8 (2.2.1), were taken from liquid cultures at an OD_{600} of 2.5 (a cell density at which BclB was seen to be well expressed, Fig. 22). One part of the cells was subsequently fixed with para-formaldehyde, washed, and incubated with the labelled antibodies (2.3.5). The remaining cells were first exposed to triton to permealize cell membranes (2.3.5), then washed, fixed and labelled as before. This step was used as a control to enable antibody binding to intracellular BclB. The lectin-deficient mutant H111-*bclACB* was also included as a control and cells from two independent experiments were analysed. As determined by confocal laser scanning microscopy, we observed that cells of the H111 parent strain were red fluorescent (Fig. 23A), whereas lectin-deficient H111-*bclACB* cells did not emit red light (Fig. 23B). Samples of H111 (pBAH8) which were treated with triton showed more red fluorescence than those that were not treated with the detergent. Due to the overlap of the GFP signal with the signal generated by the Alexa Fluor® dye, cells of the H111 wild type appeared yellow, whereas those cells of mutant H111-*bclACB* were only green fluorescent. These results suggest that BclB is associated with the bacterial cell surface. The fluorescence seemed to be equally distributed over the cell surface, implying that BclB is not localized at a particular site on the H111 cell membrane.

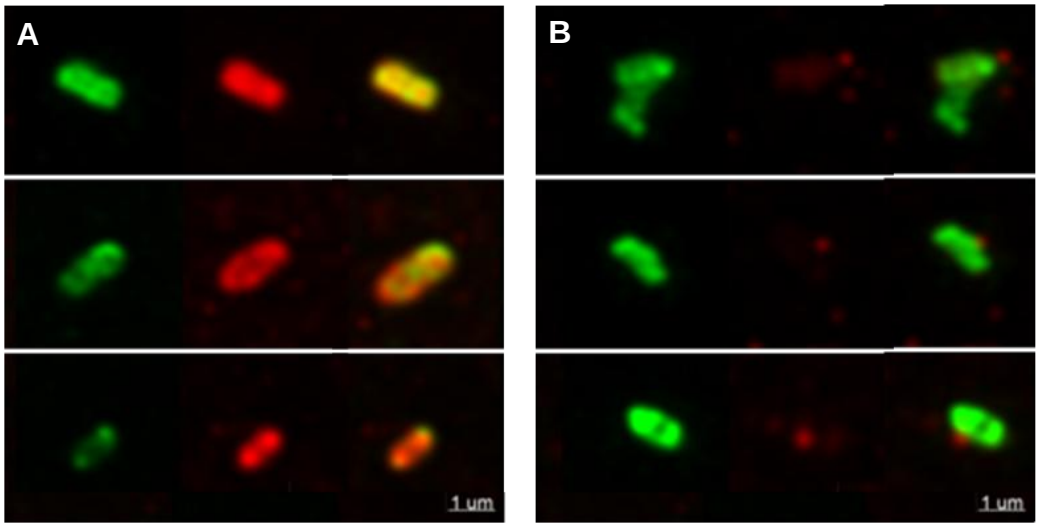


Fig. 23. Binding of Alexa Fluor® 594-conjugated BclB antibodies to the cell surface of GFP-fluorescent *B. cenocepacia* H111. The *B. cenocepacia* H111 wild type strain (**A**) and the lectin-deficient mutant H111-*bclACB* (**B**) were tagged with the *gfp*-expressing plasmid pBAH8 and grown in LB-medium to an OD_{600} of 2.5, a cell density at which BclB expression was found to be maximal. Cells were fixed and incubated with Alexa Fluor® 594-labelled antibodies directed against lectin BclB (2.3.5). Samples were analysed by determining the emission of green (left images) or red (middle images) fluorescence by confocal laser scanning microscopy. The overlay of green and red fluorescence is depicted in the images to the right.

3.4 Project IV: Characterization of the RsaM regulator

A previous screening of a *B. cenocepacia* H111 transposon library for mutants defective in *C. elegans* pathogenicity led to the identification of mutant R26. Strain R26 not only exhibited a reduced virulence but also produced increased amounts of AHL molecules. It was determined that the miniTn*5*-transposon had inserted in the intergenetic region between ORFs of *cepR* and *cepI*, 9 bp upstream of the annotated ORF BCAM1869 (Fig. 24), (Wopperer, 2008, Dissertation, University of Zurich). BCAM1869, designated *rsaM* (see 4.1.1, Discussion), is predicted to encode a protein of unknown function. To investigate the function of RsaM in the QS circuitry and in virulence of *B. cenocepacia* H111 a series of experiments were performed. Mutant R26 was initially characterized by phenotypic investigations including the analysis of the following QS-regulated functions: swarming motility, biofilm formation in polystyrene microtitre plates, the production of extracellular proteases and siderophores, pathogenicity in *C. elegans* and the qualitative detection of AHL molecules via cross-streaking. Subsequently, the transcriptome of mutant R26 was determined using two-color microarrays. To exclude any polar effects of the transposon insertion on the expression of *cepR*, a defined *rsaM* deletion mutant, named H111-rsaM, was constructed and subjected to the same phenotypic characterization as strain R26. Complementation assays were performed employing plasmid pBBR*rsaM*, containing a wild type *rsaM* allele and, as a control, plasmid pBBR*rsaM*_FS encoding the *rsaM* gene with a frameshift in the DNA sequence (2.2.5). To determine if RsaM affects the expression or the activity of the transcriptional regulators CepR or CepR2 (see Project II, 3.2), mutants R26 and H111-rsaM were also complemented with plasmid pBBR-cepR harbouring the H111 *cepR* gene as well as with plasmid pJTR2 carrying the H111 *cepR2* gene (2.2.5).

3.4.1 RsaM affects the QS circuitry and encodes a protein of unknown function

As depicted in Fig. 24, the H111 *rsaM* gene (BCAM1869, 444 bp) is divergently transcribed from *cepR* and orientated in the same direction as the *cepI* gene. The two putative starting methionine codons of CepR and RsaM are separated by 73 bp. BCAM1869 is conserved among Bcc species and is present in other proteobacteria as *Pseudomonas fuscovaginae* or *Thiomonas sp*., where it is in all cases located upstream of an AHL synthase-encoding gene (see 4.1.1, Discussion). The function of RsaM is not known, and was also not predictable by the inspection of RsaM or RsaM homologues for conserved domains in current protein databases (2.2.3). The analysis of the H111-R transcriptome (3.1, Table 5 and Fig. 6) revealed that transcript levels of *rsaM* were reduced (-6.1-fold) in the *cepR* mutant, indicating that

transcription of *rsaM* is induced by the CepIR system. These findings are in agreement with the identification of a putative *cep* box located upstream of a *-35*-consensus sequence in the *rsaM* promoter (Fig. 24). The close vicinity of the *rsaM* promoter elements to the predicted translational start site led us to question if the annotated ORF does in fact encode a protein, even though sequence analysis of *rsaM* did not predict any secondary structure motifs, as it is characteristic for sRNAs. The observation that plasmid pBBR*rsaM* carrying a wild type *rsaM* gene, but not plasmid pBBR*rsaM*_FS which encodes a frameshift-mutated *rsaM* sequence (2.2.5) was able to restore mutant phenotypes (3.4.3), suggests that *rsaM* encodes a functional protein. Since sequence inspection (2.2.3) of RsaM did not reveal a DNA-binding domain, the possibility that RsaM might function as a transcriptional regulator of genes was excluded.

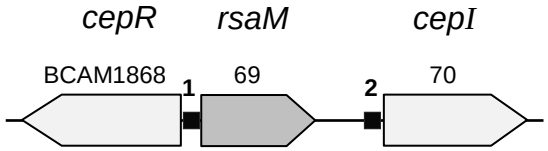


Fig. 24. Genetic organisation of the *Burkholderia cenocepacia cepR* (BCAM1868), *rsaM* (BCAM1869) and *cepI* (BCAM1870) genes. DNA sequences of *cep* boxes located within promoter regions of *cepI*, and within intergenetic regions of *cepR* and *rsaM* are GCTGTCATACTTGTCAGGT (1) and CCTGTCAAATTTATCAGTT (2).

3.4.2 Phenotypic characterization and transcriptome analyses of mutant R26

Characterization of transposon insertion mutant R26

To determine if RsaM is involved in QS regulation, mutant R26 was analysed with respect to several QS-regulated phenotypes. Two observations appeared most striking: Firstly, the various CepR-activated functions, as shown in Table 8 (3.4.3), were negatively affected so that R26 displayed in many cases a behaviour similar but not identical to that of the *cepR* mutant H111-R. Secondly, R26 activated the AHL reporter strains *Chromobacterium violaceum* CV026 and *Pseudomonas putida* F117 (pAS-C8) (2.4.2.1) to greater extents than the wild type (Fig. 30, 3.4.3), suggesting that R26 produces increased levels of AHL molecules.

The decline of the above specified CepR-induced functions in strain R26 led to the speculation that transcription of *cepR* might be diminished, possibly due to polar effects of the inserted transposon. This assumption was found to be, at least partly, true, since mutant R26 could successfully be complemented with *cepR* in *trans* with regard to the production of proteases and pathogenicity in *C. elegans* (Fig. 27 and Fig. 28, 3.4.3). To understand the extent to which the behaviour of R26 is triggered by the inactivation of *rsaM* or rather results

from an impaired transcription of *cepR*, we analysed the transcriptome of transposon insertion mutant R26 and compared it with the one of mutant H111-R (Wopperer, 2008, Dissertation).

The R26 transcriptome suggests QS-dependent expression of genes

The transcriptome analysis of transposon insertion mutant R26 was assayed employing two-color microarrays analogous to previously described methods, and is presented in detail in the thesis of Wopperer *et al.* (Wopperer, 2008). Genes and ORFs whose expression was ≥3-fold reduced in mutant R26 as compared to wild type are depicted below (Table 7).

Gene no. [a]	Description [a]	Fold change [b]
BCAS0292	conserved hypothetical protein	-105.9 *
BCAM2232	putative pyochelin biosynthetic protein PchD	-101.6 *
BCAS0293	nematocidal protein AidA	-96.8 *
BCAM2233	putative pyochelin biosynthetic protein PchC	-94.5 *
BCAM0194	conserved hypothetical protein	-79.8 *
BCAM0193	conserved hypothetical protein	-70.8 *
BCAM0192	conserved hypothetical protein	-64.3 *
BCAM2234	putative pyochelin biosynthetic protein PchB	-54.9 *
BCAM0196	conserved hypothetical protein	-47.2 *
BCAM2224	putative pyochelin receptor FptA	-45.0 *
BCAM2235	putative pyochelin biosynthetic protein PchA	-36.1 *
BCAL3285	flavohemoprotein	-33.1 *
BCAM0195	putative non-ribosomal peptide synthetase	-28.5 *
BCAM0190	put. aminotransferase - class III	-22.3 *
BCAM2230	dihydroaeruginoic acid synthetase PchE	-22.1 *
BCAM0191	put. non-ribosomal peptide synthetase	-11.3 *
BCAM0186	BclA lectin	-10.7 *
BCAM2223	putative iron uptake protein	-10.3 *
BCAM0811	putative aromatic oxygenase	-10.1
BCAL3178	LysR family regulatory protein	-9.6 *
BCAM0028	putative FHA-domain protein	-9.2 *
BCAM2222	putative membrane protein	-9.0 *
BCAM2684	putative acetyltransferase	-9.0
BCAM0810	putative aromatic oxygenase	-9.0
BCAM0189	AraC family regulatory protein	-8.9
BCAS0409	zinc metalloprotease ZmpA	-8.9
BCAM1869	conserved hypothetical protein	-8.7 *
BCAM2307	zinc metalloprotease ZmpB	-8.6 *
BCAL1677	putative type-1 fimbrial protein	-8.6
BCAM2685	conserved hypothetical protein	-8.6
BCAM2227	putative pyochelin biosynthetic protein PchG	-8.5 *
BCAM0200	efflux system transport protein	-6.9 *
BCAL2246	histidine ammonia-lyase	-6.3
BCAL1681	putative exported protein	-6.1
BCAM2142	transport system outer membrane protein	-5.8 *
BCAL1678	putative outer membrane usher protein precursor	-5.7
BCAL1813	multidrug efflux system outer membrane protein	-5.6 *
BCAM0166	NADH dehydrogenase	-5.5
BCAM0695	putative lipoprotein	-5.4 *
BCAM2226	ABC transporter ATP-binding	-5.3 *
BCAM0188	N-acylhomoserine lactone dependent regulatory	-5.2 *
BCAM0476	hypothetical protein	-5.2 *
BCAL1731	Major Facilitator Superfamily protein	-5.1
BCAS0236	putative haemagglutinin-related autotransporter	-5.0
BCAL1680	putative type-1 fimbrial protein	-4.8
BCAM1745	putative Mg-transporting ATPase	-4.8
BCAS0498	muconate cycloisomerase I 2	-4.7
BCAS0154	conserved hypothetical protein	-4.6
BCAM1927	putative exported protein	-4.5
BCAM1871	conserved hypothetical protein	-4.5 *
BCAM2141	ABC transporter protein	-4.5 *
BCAL0510	conserved hypothetical protein	-4.4
BCAS0613	AraC family regulatory protein	-4.3 *
BCAM0950	lipase chaperone	-4.3
BCAL0660	biotin carboxylase	-4.1
BCAL0659	allophanate hydrolase subunit 1	-4.1
BCAL2244	urocanate hydratase	-4.1
BCAL2353	putative sulfate transporter	-4.0 *
BCAL2791	putative kynureninase	-3.9
BCAL0358	metallo peptidase, family M1	-3.9
BCAM2720	putative phospholipase C	-3.9
BCAL3179	probable D-lactate dehydrogenase	-3.8 *
BCAM1811	conserved hypothetical protein	-3.8 *

BCAL2451	putative membrane protein	-3.8
BCAM2514	putative fatty aldehyde dehydrogenase	-3.8 *
BCAM1713	putative ionic antiporter	-3.8
BCAM0634	hypothetical protein	-3.8
BCAL2450	putative metal transporter	-3.8 *
BCAM0805	muconate cycloisomerase I 1	-3.7
BCAL0852	conserved hypothetical protein	-3.7 *
BCAL2241	putative hlorohydrolase	-3.7
BCAM0030	conserved hypothetical protein	-3.7
BCAM1870	N-acylhomoserine lactone synthase CepI	-3.7*
BCAS0155	conserved hypothetical protein	-3.6
BCAM1413	conserved hypothetical protein	-3.6
BCAM0185	BclC lectin	-3.5 *
BCAM0184	BclB lectin	-3.5 *
BCAL1249	putative PHB depolymerase	-3.5 *
BCAM0392	putative acetyltransferase	-3.4 *
BCAL1921	conserved hypothetical protein	-3.4
BCAL2243	conserved hypothetical protein	-3.4
BCAM2153	putative cytochrome P450 oxidoreductase	-3.4
BCAL0744	Appr-1-p processing enzyme family protein	-3.4 *
BCAL0831	putative storage protein	-3.4
BCAM2215	putative copper resistance protein C precursor	-3.4
BCAM0491	TonB-dependent receptor	-3.3
BCAM1706	putative membrane protein	-3.3 *
BCAM1005	putative acyltransferase	-3.3
BCAM2429	3 putative lipoprotein	-3.3 *
BCAL3187	putative oxidoreductase	-3.3
BCAL1752	conserved hypothetical protein	-3.2
BCAL0661	putative biotin-binding protein	-3.2
BCAL0552	allophanate hydrolase subunit 1	-3.2
BCAL1524	putative lipoprotein	-3.2
BCAS0636	conserved hypothetical protein	-3.2
BCAL2980	putative oxygenase	-3.2
BCAL0121	aquaporin Z	-3.2
BCAM2308	putative leucyl aminopeptidase precursor	-3.2
BCAM2231	transcriptional regulator PchR	-3.2
BCAM2444	putative exported protein	-3.1
BCAL2734	conserved hypothetical protein	-3.1
BCAL0662	LamB/YcsF family protein	-3.1
BCAM2055	type III secretion system protein	-3.1
BCAL0431	conserved hypothetical protein	-3.1
BCAM1554	putative diguanylate cyclase	-3.1
BCAM0633	conserved hypothetical protein	-3.0
BCAL1268	dihydropteroate synthase	-3.0
BCAL2245	putative histidine utilization repressor	-3.0
BCAM0804	catechol 1,2-dioxygenase 1	-3.0
BCAM0393	putative beta-lactamase, class D	-3.0
BCAM2216	putative exported protein	-3.0
BCAL3136	bis(5'-nucleosyl)-tetraphosphatase, symmetrical	-3.0
BCAM2143	cable pilus associated adhesin protein	-3.0
BCAL0343	conserved hypothetical protein	-3.0
BCAM2062	conserved hypothetical protein	-3.0
BCAL1753	LysR family regulatory protein	-3.0
BCAM2482	agmatinase	-3.0
BCAM2426	putative diguanylate phosphodiesterase	-3.0

Table 7. Differentially regulated genes and ORFs in the transposon insertion mutant R26. Listed are genes (nomenclature according to Holden *et al.* (2009); a), whose expression is at least 3-fold reduced in the R26 transcriptome as compared to wild type; b. Genes and ORFs which were also seen regulated by CepR in a previous transcriptome analysis (3.1) are highlighted in grey. P-values (<0.05) of significantly regulated genes are indicated by an asterisk.

Notably, the transcription of 61 of the 118 listed genes (highlighted in grey) has previously been shown to be induced by CepR (Table A1 and A2, Appendix). These findings are in agreement with the observation that transcript levels of *cepR* (BCAM1868) are reduced in the R26 transcriptome (-2.8-fold) (Wopperer, 2008). Importantly, as transcript levels of *cepI* (BCAM1870) are only marginally decreased in the R26 transcriptome (-3.7-fold) relative to the expression changes that were observed in the transcriptome of the *cepR* mutant H111-R (-28-fold), it remains unclear to which extent the transcription of *cepR* is affected in mutant R26. The fact that both phenotypes of R26 the ability to cause infection in *C. elegans*, and the production of extracellular proteases could be rescued by providing *cepR* in *trans*, supports

the hypothesis that expression or activity of CepR is diminished in mutant R26 (Fig. 27 and Fig. 28, 3.4.3).

The R26 transcriptome comprises highly regulated genes which were not identified in the H111-R transcriptome, indicating that transcription of these genes may be regulated by RsaM (Table 7), (Wopperer, 2008). These genes include amongst others: BCAM2232-35 encoding pyochelin biosyntheses genes, BCAM2224 encoding a putative pyochelin receptor protein FptA, and BCAM2230 encoding the dihydroaeruginoic acid synthetase PchE.

3.4.3 Phenotypic characterization of mutant H111-rsaM and complementation

To unambiguously investigate the role of RsaM in global gene regulation in *B. cenocepacia* H111, a defined *rsaM* deletion mutant was constructed. This mutant, as well as the transposon insertion mutant R26, was compared with the H111 wild type strain. Complementation of both mutants with *rsaM* was achieved employing plasmid pBBR*rsaM* (2.2.8). To determine if RsaM influences the expression or activity of CepR or CepR2, mutants were complemented with the respective wild type genes (2.2.1). The expression of the investigated phenotypic traits was compared with the behaviour of the wild type as summarized below (Table 8).

Phenotypic trait	**H111-R**	**R26**	**H111-rsaM**
Swarming motility [a)]	- - -	- -	-
Biofilm formation [b)]	- - -	- -	-
Protease production [c)]	- - -	- -	-
Pathogenicity in *C. elegans* [d)]	- - -	- -	/ (-)
Siderophore production [e)]	- - -	- -	-
Production of C8-HSL/ C6-HSL [f)]	- - -	+	+

Table 8. Expression of QS-activated functions in the H111 mutants H111-R, R26 and H111-rsaM in comparison to the wild type. Phenotypic traits of the *cepR* mutant, mutant R26 and the *rsaM* mutant H111-rsaM are reduced (-), increased (+), or unchanged/ marginally reduced (/ (−)), in comparison to the wild type.

[a)]: Swarming mortility was analysed as described in 2.4.5 and illustrated in Fig. 25.
[b)]: Biofilm formation was assayed in polysterol microtitre plates and bacterial strains were grown in AB minimal medium supplemented with 10 mM sodium citrate (2.4.6.1 and Fig. 26).
[c)]: The production of extracellular proteases was identified employing milk-agar plates (2.4.3 and Fig. 27).
[d)]: The pathogenic potential was examined employing the *C. elegans* slow-killing assay (2.4.8.1 and Fig. 28).
[e)]: CAS agar plates were employed for the detection of siderophore activities (2.4.4 and Fig. 29).
[f)]: The production of AHL molecules was analysed via cross-streaking (2.4.2.2 and Fig. 30), by TLC (2.4.2.5 and Fig. 35), and along the bacterial growth curves employing AHL sensor strains (2.4.2.1 and Fig. 36).

Mutant H111-rsaM exhibited a reduced swarming motility on AB medium supplemented with sodium citrate (Fig. 25), and was impaired in biofilm formation when bacteria were grown in polystyrene microtitre plates (Fig. 26). Swarming behaviour of the *rsaM* mutant could be restored when *rsaM* was provided in *trans*, and AHL molecules were externally provided.

Importantly, the presence of plasmid pBBR-cepR in R26 (data not shown) and mutant H111-rsaM (Fig. 25) did not restore the motility of the strains, indicating that the effect of RsaM on swarming motility is CepR-independent. AHL molecules were added to the medium, because *rsaM* overexpression strongly decreased the production of AHL molecules in H111 (see 3.4.5). Biofilm formation of mutant H111-rsaM was also rescued in the presence of pBBR*rsaM*. Surprisingly, this was possible without the external addition of AHLs (Fig. 26). Given the fact, that the presence of the empty plasmid pBBR1MCS in mutant H111-rsaM also resulted in increased biofilm formation (Fig. 26), it seems most likely that the elevated biofilm formation of strain H111-rsaM (*rsaM*$^{+}$) was only triggered by the cloning vector.

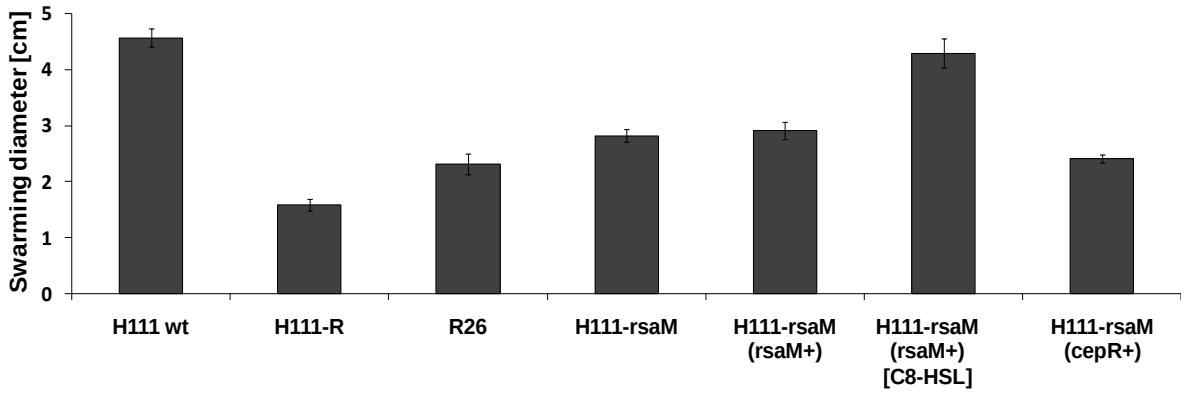


Fig. 25. Swarming activities of the *B. cenocepacia* H111 wild type, mutants and complemented strains. Bacterial cultures of the wild type (wt), the *cepR* mutant H111-R, the transposon insertion mutant R26, the *rsaM* mutant H111-rsaM, as well as the *cepR* and *rsaM* complemented mutant, were spotted on AB minimal medium solidified with 0.4% agar and supplemented with 10 mM sodium citrate and 0.1% casamino acids (2.2.2). The motility was determined after incubation of 48h at 30°C (2.4.5). The *rsaM* complemented strain H111-rsaM (*rsaM*$^{+}$) was also examined in the presence of 0.2 µM *N*-octanoyl-homoserine lactone (C8-HSL). Error bars are values ± the standard error of the mean of $n = 4$.

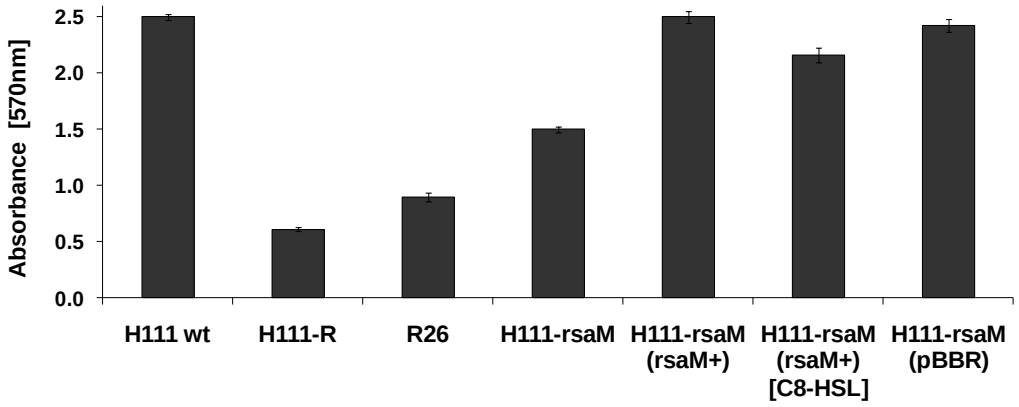


Fig. 26. Biofilm formation of the *B. cenocepacia* H111 wild type, QS mutant and complemented strains. The *B. cenocepacia* H111 wild type (wt), the *cepR* mutant H111-R, the transposon insertion mutant R26, the *rsaM* mutant H111-rsaM, as well as the *rsaM* complemented mutant, were grown in polystyrene microtitre dishes using buffered AB medium (pH 7) supplemented with 10 mM sodium citrate. Biofilm formation of the *rsaM* complemented H111-rsaM mutant was also examined in the presence of 0.2 µM *N*-octanoyl-homoserine lactone (C8-HSL). Strain H111-rsaM was also complemented with plasmid pBBR1MCS (pBBR) as a vector control. After the incubation for 48h at 30°C planktonic cells were removed and attached cells were stained with crystal violet as described (2.4.6.1). Error bars represent the standard error of the mean of six independent wells.

In contrast to mutant R26 we observed that the expression of some QS-induced phenotypes was only weakly affected in mutant H111-rsaM (Table 8), including the production of extracellular proteases (Fig. 27) and the production of siderophores (Fig. 29). Moreover, mutant H111-rsaM exhibited a slight reduction of virulence in the *C. elegans* infection model when compared to the wild type. However, these differences were not statistical significant with $n = 4$ (Fig. 28).

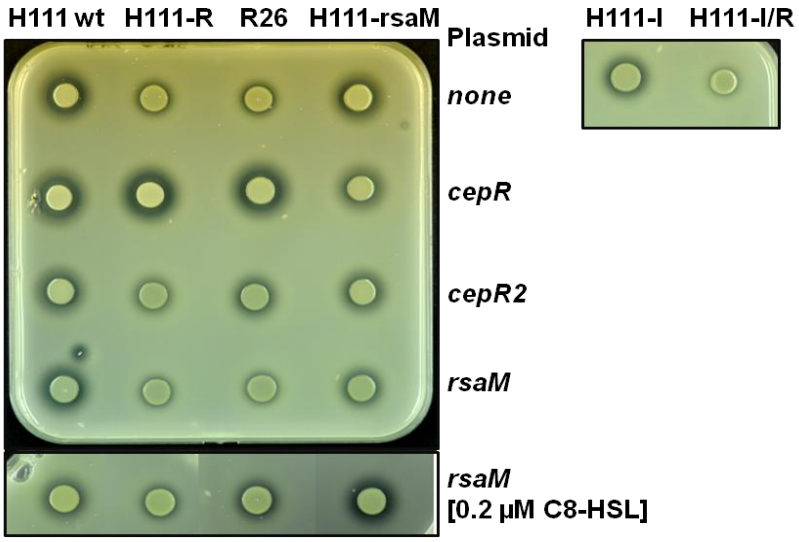


Fig. 27. Protease activities of the *B. cenocepacia* H111 wild type, mutants and complemented strains. The *B. cenocepacia* H111 wild type (wt), the *cepR* mutant H111-R, the transposon insertion mutant R26, the *rsaM* mutant H111-rsaM, as well as the *cepI* mutant H111-I and mutant H111-I/R (2.1.1) which has a deletion in all three genes *cepR*, *rsaM* and *cepI* were analysed for the production of extracellular proteases on milk-agar plates (24h at 30°C, 2.4.3). Complementation of strains was achieved by providing *cepR* (pBBR-cepR), *cepR2* (pJTR2) or *rsaM* (pBBR*rsaM*) in *trans* (2.2.1). *RsaM* complemented strains were additionally analysed on plates containing 0.2 µM *N*-octanoyl-homoserine lactone (C8-HSL).

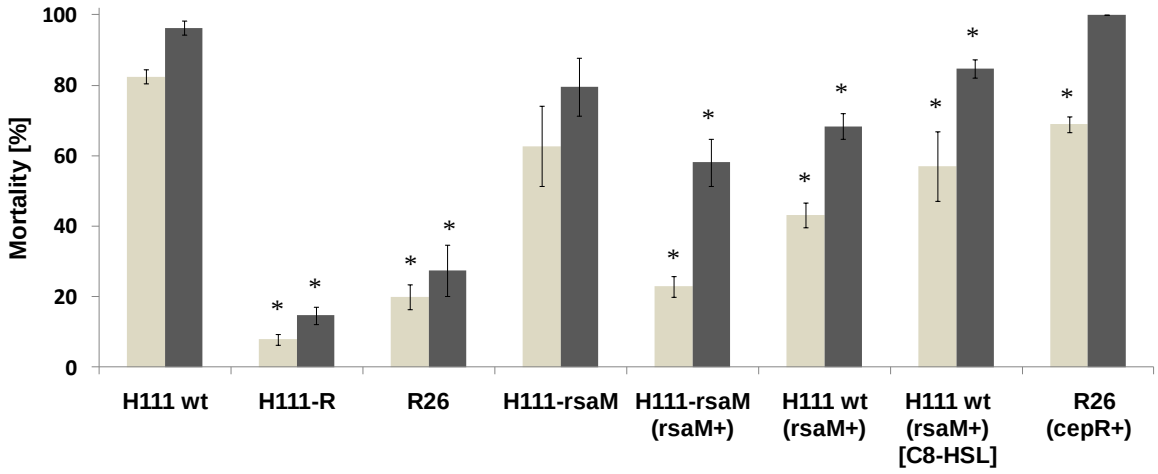


Fig. 28. Killing of nematodes by the *B. cenocepacia* H111 parent, mutants and complemented strains. *Caenorhabditis elegans* slow-killing assays (2.4.8.1) were performed employing the H111 wild type (wt), the *cepR* mutant H111-R, the transposon insertion mutant R26, the *rsaM* mutant H111-rsaM, as well as *cepR* and *rsaM* complemented mutants. The mortality of the nematodes was determined after 48h (light-shaded bars) and 72h (dark-shaded times) at 20°C. *RsaM* complemented strains were additionally analysed on NGMII-plates containing 0.2 µM *N*-octanoyl-homoserine lactone (C8-HSL). Error bars represent the standard error of the mean of $n = 4$. The asterisk indicates significant differences when compared to the wild type (Student's *t*-test, $p < 0.05$).

The following observations were made when analysing siderophore production of *B. cenocepacia* H111 strains on CAS agar (see also 3.2.2), which visualizes siderophore activities of pyochelin (violet) and of ornibactin (yellow): siderophore production was diminished in mutant R26 to a similar extent as observed in mutant H111-R, whereas the amounts of siderophores produced by mutant H111-rsaM were only marginally decreased in comparison to the wild type (Fig. 29). The complementation of R26 with *rsaM* could restore the wild type phenotype, and *rsaM* complementation also resulted in a slightly increased production of pyochelin in mutant H111-rsaM. The H111 *cepR2* mutant, by contrast, could not be complemented with plasmid pBBR*rsaM*. As expected, the presence of CepR2 led to an increased production of pyochelin in all mutant strains (see also 3.2.2).

These observations allow the following conclusions: RsaM stimulates the production of the siderophore pyochelin, and this stimulation appears to be dependent on the presence of CepR2.

Previoulsy, we have shown that the production of ornibactin is negatively regulated by CepR (3.2.2, Fig. 11B). For this reason, complementation of the wild type with *cepR* resulted in a reduced zone of activity. Interestingly, this effect was not observed with the *rsaM* mutant (Fig. 29).

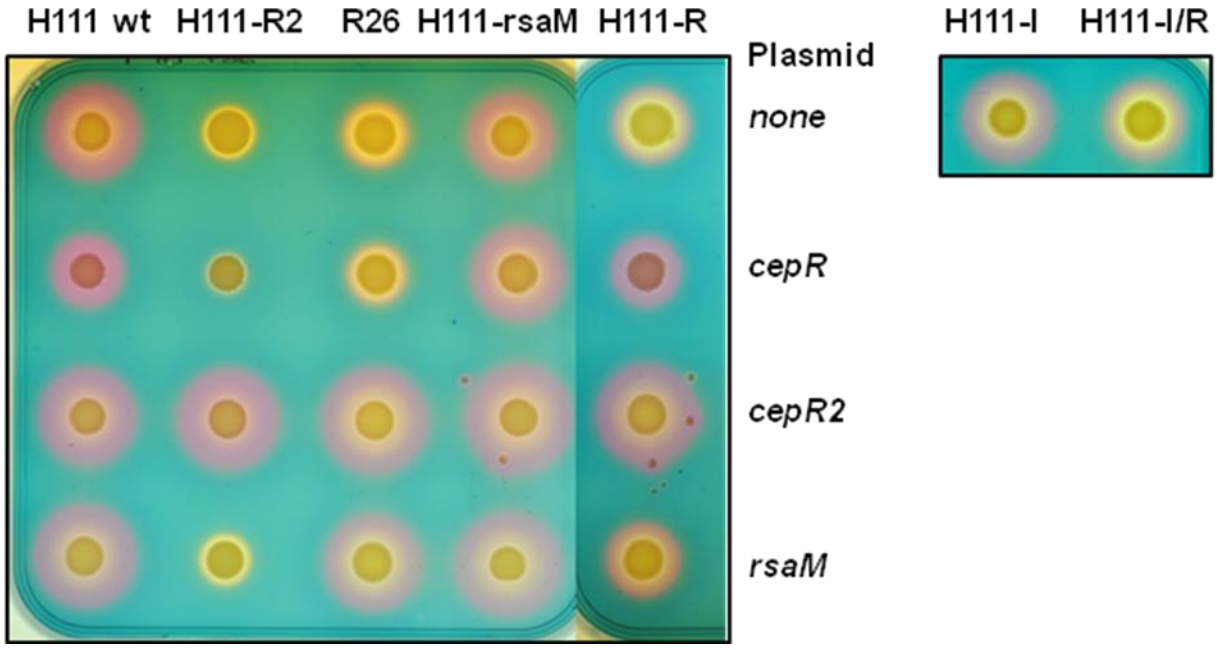


Fig. 29. CAS activities of the *B. cenocepacia* H111 wild type, mutants and complemented strains. The *B. cenocepacia* H111 wild type strain (wt), the *cepR2* mutant H111-R2, transposon insertion mutant R26, the *rsaM* mutant H111-rsaM, the *cepR* mutant H111-R , the *cepI* mutant H111-I and mutant H111-I/R, which has a deletion in all three genes, *cepR*, *rsaM* and *cepI* (2.1.1), were spotted on CAS agar plates and analysed for the production of siderophores after 48h at 37°C (2.4.4). Complementation of strains was achieved by providing *cepR* (pBBR-cepR), *cepR2* (pJTR2) or *rsaM* (pBBR*rsaM*) in *trans* (2.2.1).

We noticed that mutant H111-rsaM exhibited increased extracellular levels of AHL signal molecules, an observation also made in cross-streak experiments of the transposon insertion mutant R26 (Fig. 30). Notably, the production of AHL molecules was diminished when *rsaM* was provided in *trans* in strains R26 or H111-rsaM (Fig. 30 and 3.4.5).

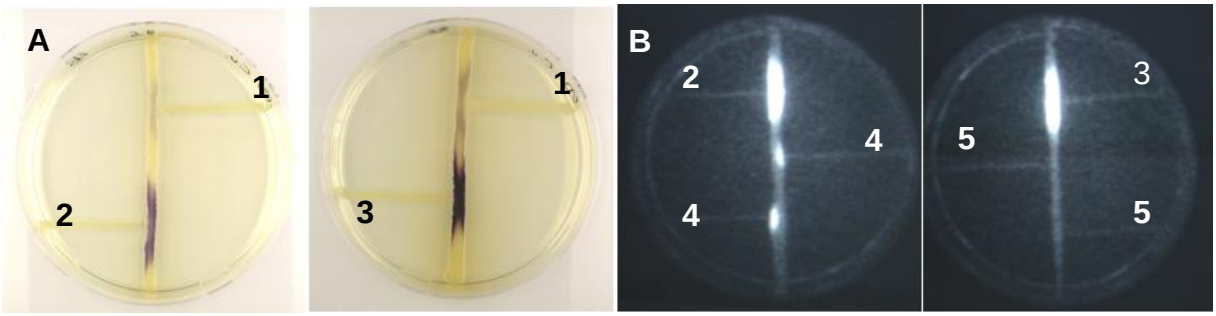


Fig. 30. Detection of AHL production of the *B. cenocepacia* H111 parent and mutants via cross-streaking. The *B. cenocepacia* H111 wild type (1), transposon insertion mutant R26 (2), the *rsaM* mutant H111-rsaM (3) and transconjugants R26 (*rsaM*$^{+}$) (4) and H111-rsaM (*rsaM*$^{+}$) (5) were streaked against reporter strains *Chromobacterium violaceum* CV026 (**A**) and *Pseudomonas putida* F117 (pAS-C8) (**B**) (2.4.2.1). Plates were analysed for the development of violacein or the expression of GFP following incubation for 24h at 30°C.

3.4.4 RsaM seems to modulate the activity of LuxR family transcriptional regulators

3.4.4.1 RsaM seems to influence the activity of CepR2

The following observations suggested that RsaM might influence the expression or the activity of the orphan LuxR homologue CepR2, which has been identified to stimulate the production of pyochelin in *B. cenocepacia* H111 (3.2): i) transcript levels of pyochelin biosynthesis genes were highly down-regulated in the transcriptome of mutant R26 in comparison to the transcriptome of the wild type (3.4.2) and ii) complementation with *rsaM* increased pyochelin production in all mutant strains except in mutant H111-R2 (Fig. 29, 3.4.3). Complementation with *rsaM* moreover enhanced the production of pyochelin in mutant R26 to similar amounts as observed in the wild type, and mutant H111-R complemented with *rsaM* exhibited an increased production of pyochelin (Fig. 29). To further investigate the impact of RsaM on the expression of pyochelin we analysed the activities of the *pchD* promoter (the first gene of the pyochelin biosynthesis operon BCAM2232 to BCAM2235) as well as of the divergently transcribed *pchR* gene (BCAM2231, encoding a transcriptional regulator of pyochelin production) in the mutant background R26 and H111-rsaM. Furthermore, we determined the CAS activities of pyochelin extracts of the H111 mutants and their *rsaM* complemented derivates.

The microarray profiling revealed that pyochelin biosynthesis genes were down-regulated to a greater extent in the transcriptome of mutant R26 than in the transcriptome of mutant

H111-R. Regulated genes comprised BCAM2232 to 35, a gene cluster required for pyochelin biosynthesis, which was not at all differentially regulated in the H111-R transcriptome (Table 7, 3.4.2). These observations suggested that RsaM might influence the expression of pyochelin by regulating the expression of BCAM2232 to 35. We therefore investigated the promoter activities of *pchD* (BAM2232, driving expression of the pyochelin biosynthesis operon BCAM2232 to BCAM2235). Promoter activities were not seen to be diminished in mutant R26 or mutant H111-rsaM (data not shown). However, differences were difficult to determine, as Miller Units measured for the P_{pchD}-*lacZ* fusion (2.2.8) were generally low, independent of whether the assay was performed in LB-medium or in iron-depleted succinate medium (see 3.2.2, Fig. 12). We then analysed the transcription of the gene *pchR* (BCAM2231), encoding a transcriptional regulator of pyochelin production. The expression of *pchR* has been shown to be stimulated by CepR2 (3.2.2, Fig. 12), since promoter activities of P_{pchR}-*lacZ* (2.2.8) are almost completely lost in mutant H111-R2 (Fig. 31). As before, *pchR* promoter activities were not decreased in mutant R26 or in mutant H111-rsaM in comparison to the activities observed in the wild type when strains were grown in LB-medium (Fig. 31). Miller Units of R26 and H111-rsaM also resemble those of the wild type when strains were cultured in succinate medium (data not shown).

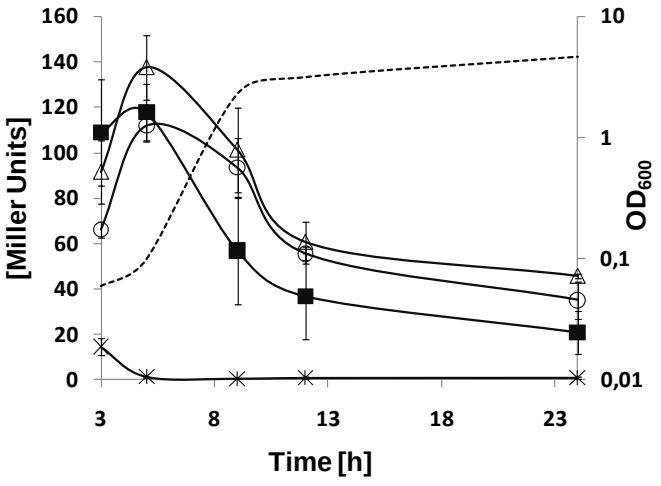


Fig. 31. Promoter activities of *pchR* are not reduced in mutant H111-rsaM. Activities of the *pchR* (BCAM2231) promoter driving expression of one of the pyochelin biosynthesis operons were determined when strains were grown in LB-medium. Growth (dashed line, wild type) and β-galactosidase activities of the H111 wild type (closed rectangles), the transposon insertion mutant R26 (open triangle), the *rsaM* mutant H111-rsaM (open circles) and the *cepR2* mutant H111-R2 (stars) were monitored throughout the growth curve. The values are means ± the standard error of the mean (n = 3).

To further determine the impact of RsaM on the expression of pyochelin, we also extracted pyochelin from liquid cultures of the H111 parent, mutant, and the *rsaM* complemented strains, and analysed the CAS activities as described before (2.4.4). However, pyochelin extracts showed so little activity that no firm conclusion could be made (data not shown).

3.4.4.2 RsaM seems to indirectly affect the transcription of CepR-regulated genes

We observed that mutant H111-rsaM was impaired in the ability to swarm and to form a biofilm (3.4.3, Fig. 25 and 26). CAS activities of transconjugants R26 (*cepR*$^+$) and H111-rsaM (*cepR*$^+$) suggested that the function of CepR might be dependent on the presence of RsaM (3.4.3, Fig. 29). To address this issue, we determined the β-galactosidase activitities of CepR-activated promoters (*cepI* and *aidA*) in the mutant background R26 and H111-rsaM, and compared the activities with those obtained in the H111 wild type. As depicted in Fig. 32A, promoter activities of *cepI* (fusion P$_{cepIlong}$-*lacZ*, 2.2.8) were approximately 10-fold reduced in both mutant strains. We also determined the activity of the P$_{cepIlong}$-*lacZ* fusion in the wild type strain amended with AHL molecules (C8-HSL) to ensure that transcription is not affected by the increased levels of AHL molecules in strains R26 and H111-rsaM (3.4.5). However, the addition of signal molecules to the medium did not influence promoter activities in the wild type strain (data not shown). When analysing the activity of the *aidA* promoter (promoter fusion P$_{aidA1}$-*lacZ*, 2.2.8) similar expression trends were observed (Fig. 32B). Here, Miller Units were reduced by approximately 50% in the exponentially growth phase in the *rsaM* mutant relative to the wild type. After 24h of growth the activities of P$_{aidA1}$-*lacZ* detected in the wild type and mutant H111-rsaM were found to be similar.

It is important to note, that the orphan LuxR homologue CepR2 was previously found to be important for full activity of the *aidA* promoter (Toller, 2008). Even though CepR2 did not activate the H111 *cepI* promoter in an *E. coli* background (3.2.4), it cannot be excluded that the decreased promoter activities of *cepI* and *aidA* in R26 and H111-rsaM are due to lowered CepR2 levels or altered CepR2 activity.

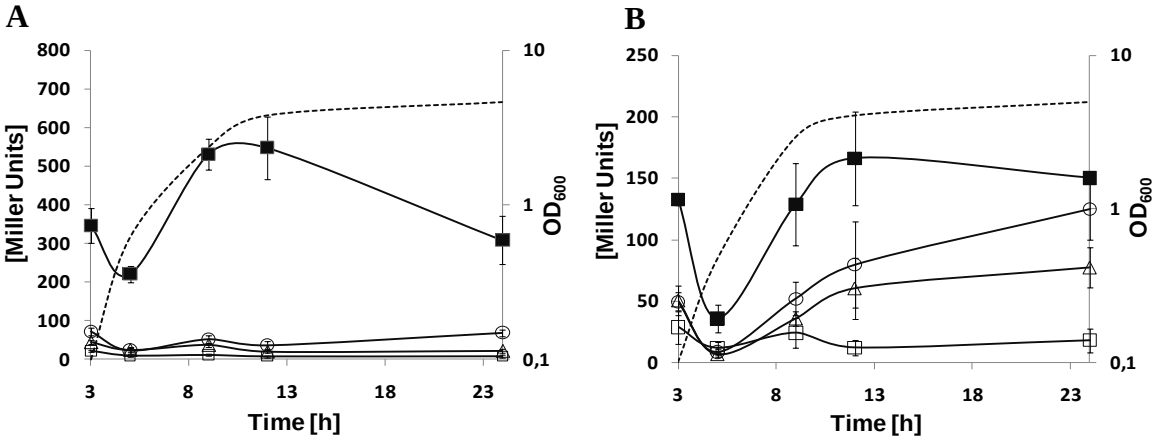


Fig. 32. Activities of the *cepI* promoter (A) and the *aidA* promoter (B) are dependent on RsaM. The transcription of the *cepI* gene (promoter fusion P$_{cepIlong}$-*lacZ*) and of *aidA* (fusion P$_{aidA}$-*lacZ*) was determined in the H111 wild type (wt, closed rectangles), the *cepR* mutant H111-R (open rectangles), the R26 transposon insertion mutant (open triangles) and in the *rsaM* mutant H111-rsaM (open circles). β-galactosidase activities were monitored in LB-medium throughout the growth curve (dashed line, wt). Values are means ± the standard error of the mean (n = 3).

The effect of RsaM on the activity of CepR was further investigated by employing the *cep*-based reporter plasmid pAS-C8 (2.4.2.1) in different strain backgrounds (H111 wild type and mutants H111-I, H111-I/R and H111-rsaM). The activity of CepR was considered to be directly proportional to the GFP fluorescence. To this end, the various strains were grown for 48h at 30°C in the presence or absence of C8-HSL. As depicted in Fig. 33, expression of GFP was only observed in cultures of the H111 wild type and the H111-rsaM mutant when no signal molecules were added to the medium. Moreover, strain H111-rsaM (pAS-C8) exhibited an approximately 25% increased fluorescence relative to the wild type strain harbouring the sensor plasmid, most likely because of the elevated AHL levels produced by the *rsaM* mutant (3.4.5). The addition of C8-HSL led to an activation of the reporter plasmid in the *cepI* mutant H111-I and in mutant H111-I/R, in which all three genes *cepR*, *rsaM* and *cepI* are deleted (2.1.1). Even though the expression of GFP constituted only about 2/3 of the RFU determined in the wild type, we observed a similar activation of the reporter plasmid in the mutant background of H111-I or H111-I/R when C8-HSL were provided to the medium. These observations suggest that RsaM does not stimulate the activity of CepR, at least under the conditions used in this assay.

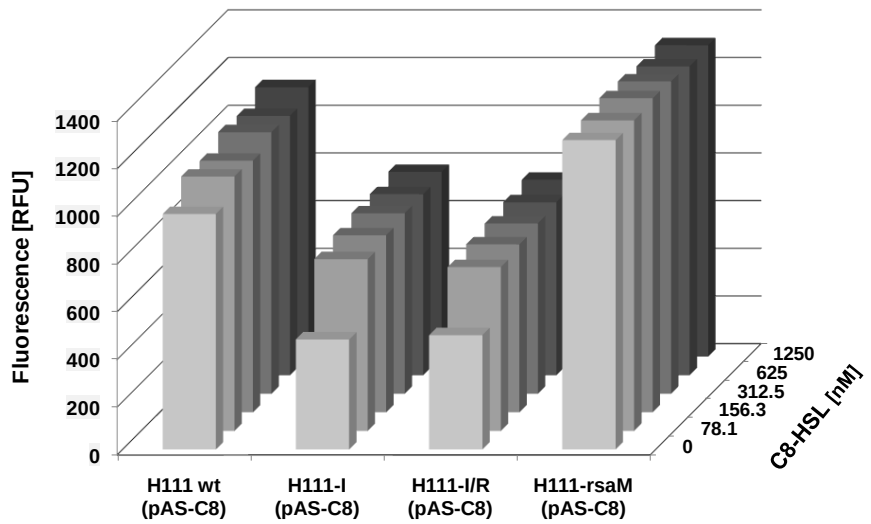


Fig. 33. RsaM does not seem to stimulate the CepR-mediated activation of a P_{cepI}-*gfp* transcriptional fusion. The expression of GFP (RFU, relative fluorescence units) of the AHL biosensor pAS-C8 (2.4.2.1) was measured in the H111 wild type (wt), the *cepI* mutant H111-I, mutant H111-I/R, in which all three genes, *cepR*, *rsaM* and *cepI* are deleted, and in the *rsaM* mutant H111-rsaM (2.1.1). Measurements were performed in the presence of different concentrations of *N*-octanoyl-homoserine lactone (C8-HSL) after incubation for 48h at 30°C.

3.4.4.3 RsaM seems to modulate the protein activity of LuxR

The observation that RsaM may modulate the activity of the orphan LuxR homologue CepR2 (3.4.4.1) raised the question if the protein might function as an effector of LuxR-like proteins. To investigate if RsaM influences the activity of the *Vibrio fischeri* LuxR transcriptional regulator, plasmid pBBR*rsaM* and, as a control, plasmid pBBR*rsaM*_FS encoding a frameshifted *rsaM* sequence (2.2.1) were transferred into *E. coli* MT102 harbouring the *gfp*-expressing *lux*-based AHL sensor plasmid pJBA89 (2.4.2.1). As expected, the presence of plasmid pBBR*rsaM*_FS did not affect GFP expression of the plasmid (Fig. 34) when AHL molecules were provided: pJBA89 is based on components of the LuxIR QS system of *Vibrio fischeri*, and thus is more sensitive to 3-oxo-C8-HSL than to C8-HSL. Interestingly, when *rsaM* was provided in *trans* the expression of GFP was inhibited, and the addition of increasing concentration of AHL molecules (3-oxo-C8-HSL and C8-HSL) did not induce the expression of GFP. To exclude the possibility that the added AHL molecules are degraded we determined the AHL levels in the wells after the experiments employing *E. coli* MT102 (pJBA89). However, no inactivation could be seen, suggesting that RsaM does not function as a lactonase (Fig. 34). As it seems unlikely that RsaM acts as a transcriptional repressor (see 3.4.1), it can be speculated that RsaM may interfere with the activity of LuxR.

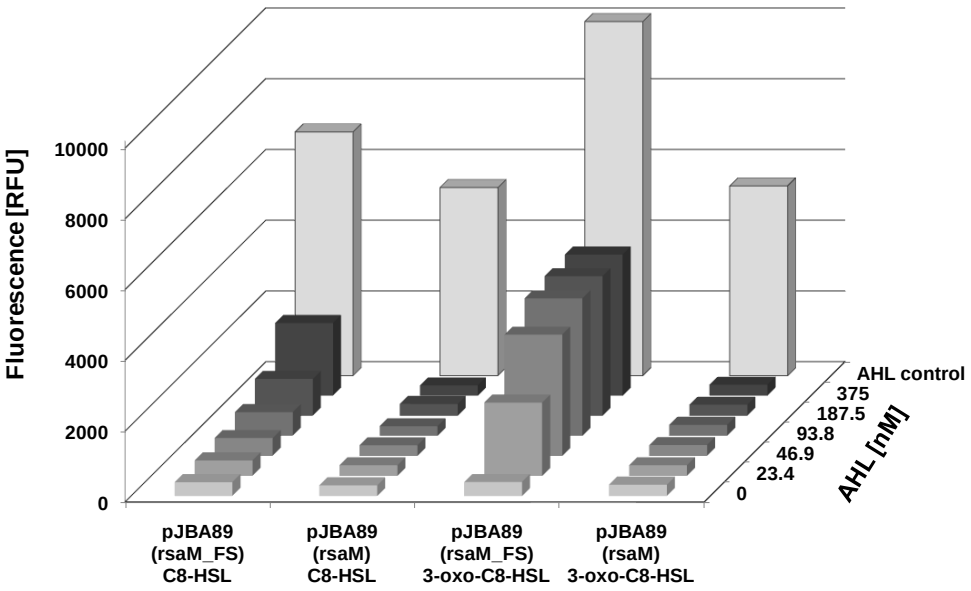


Fig. 34. RsaM seems to repress the LuxR-mediated activation of a P_{luxI}-*gfp* transcriptional fusion. The GFP expression (RFU, relative fluorescence units) of the AHL biosensor *E. coli* MT102 (pJBA89) was measured in the presence of plasmid pBBR*rsaM*_FS (rsaM_FS) encoding a frameshifted *rsaM* sequence, and plasmid pBBR*rsaM* (rsaM) encoding *rsaM* (2.2.1). Measurements were obtained in the presence of different concentrations of *N*-octanoyl-homoserine lactone (C8-HSL) and *N*-3-oxo-octanoyl-homoserine lactone (3-oxo-C8-HSL) after an incubation for 6h at 30°C. Culture samples of exponentially phase-grown *E. coli* MT102 (pJBA89) were added after the measurement to the wells with AHL concentrations of 375 nM and incubated for another 6h at 30°C to determine the AHL concentration at the end of the experiments (AHL control).

3.4.5 RsaM negatively affects extracellular levels of AHL signal molecules

Experiments employing cross-streaks of the transposon insertion mutant R26, mutant H111-rsaM and the *rsaM* complemented mutants (3.4.3, Fig. 30) suggested that RsaM negatively affects the production or the efflux of AHL molecules. The possibility that RsaM functions as a lactonase was found to be unlikely (3.2.4). As both the transposon insertion mutant R26 and the defined mutant H111-rsaM revealed the same increase in the extracellular amounts of AHL molecules in the analyses described below, the impact of RsaM on the production of AHLs will only be illustrated by the characterization of mutant H111-rsaM. To investigate if the production of both signal molecules synthesized by *B. cenocepacia* H111, *N*-hexanoylhomoserine lactone (C6-HSL) and *N*-octanoyl-homoserine lactone (C8-HSL), which are produced in a ratio of 1:10 (Gottschlich *et al*., 2001), are equally affected by RsaM, AHL molecules were extracted from bacterial cultures of the H111 *rsaM* mutant and *rsaM* complementated strains (2.4.2). The HSL were then separated by thin layer chromatography and visualized by means of the reporter plasmid pAS-C8 (2.4.2). As shown in Fig. 35, mutant H111-rsaM exhibited an at least two-fold increase in the amounts of both molecules, C6-HSL and C8-HSL. In agreement with earlier findings, the complementation of mutant H111-rsaM and the H111 parent with plasmid pBBR*rsaM* strongly impaired the production of both AHL molecules.

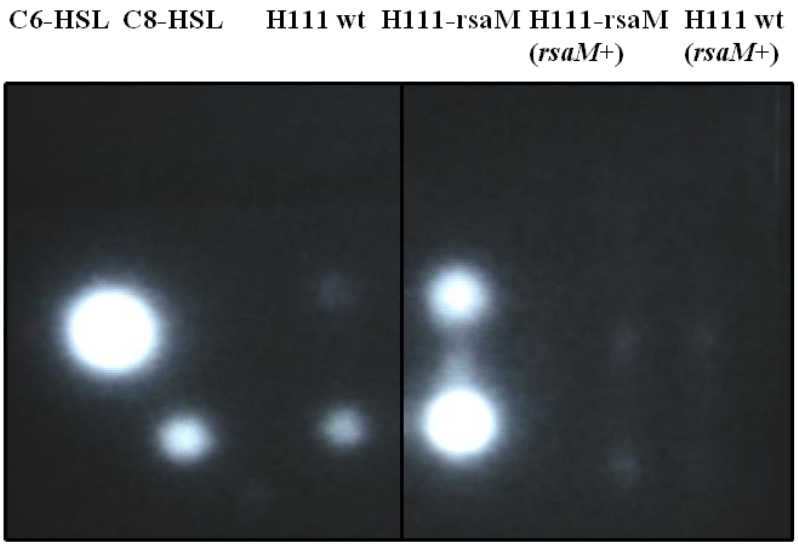


Fig. 35. Detection of AHL molecules by thin layer chromatography and AHL sensor plasmid pAS-C8. AHL molecules were extracted from supernatants of overnight cultures of the *B. cenocepacia* H111 wild type (wt), mutant H111-rsaM, and *rsaM* complemented strains, separated by RP-TLC (2.4.2.5) and detected by determining the expression of GFP from reporter strain *P. putida* (pAS-C8) (2.4.2.2). AHL standards [1 mM] are *N*-hexanoyl-homoserine lactone (C6-HSL) and *N*-octanoyl-homoserine lactone (C8-HSL).

The production of AHL molecules was also monitored along the bacterial growth curve, as shown in Fig. 36. For that purpose, the bacterial strains were cultured in wells of microtitre dishes and inoculated in LB-medium with exponentially phase-grown cells of the *gfp*-expressing reporter strain *P. putida* F117 (pAS-C8) (2.4.2.4). It was seen that the expression of GFP in those wells that were inoculated with the *rsaM* mutant was already induced in the early growth phase, exhibiting an activation peak at around 6 - 10h of growth. These results suggest that RsaM might be important for the timing of AHL production.

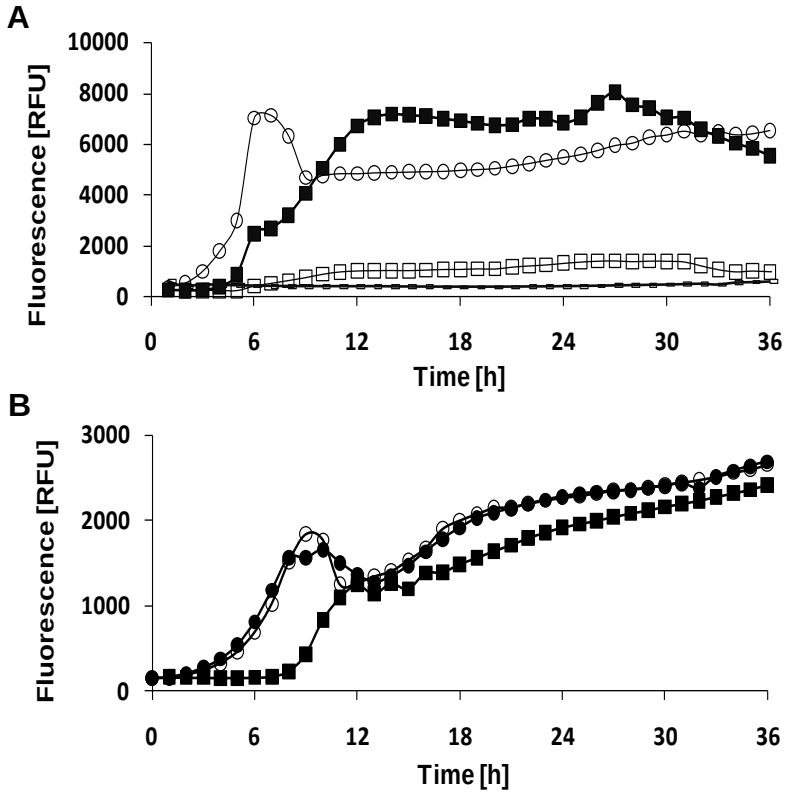


Fig. 36. Analysis of AHL production along the bacterial growth curve. Overnight cultures of the *B. cenocepacia* H111 wild type (wt, closed rectangles), the *rsaM* mutant H111-rsaM (open circles), *rsaM* complemented strains H111-rsaM (*rsaM*$^+$) (dashed line) and H111 wt (*rsaM*$^+$) (open rectangles), and mutant H111-rsaM harbouring plasmid pBBR*rsaM*_FS encoding a frameshifted *rsaM* ORF (2.2.5, closed circles) were inoculated to an OD_{600} of 0.05 in wells of microtitre dishes. Cells of exponentially-phase-grown *P. putida* F117 (pAS-C8) were added, obtaining final volumes of 300 µl. Samples were incubated for 48h at 30°C, and the expression of GFP was detected as described (2.4.2.4). Measurements derived from two independent experiments are shown (**A** and **B**).

Recently, it was observed that the QS-regulated phenotypes of the H111 *cepI* mutant could not entirely be rescued by complementation with plasmid pBBR1MCS5*cepI*, carrying the H111 *cepI* gene under the control of the *lac* promoter of the plasmid (Uehlinger, 2009, Dissertation). Since this might be caused by the negative effect of RsaM, plasmid pBBR1MCS5*cepI* was transferred into mutant H111-R, in which transcription of *rsaM* has been shown to be decreased (see 3.4.1), and in mutant H111-I/R, in which all three genes, *cepR*, *rsaM* and *cepI* are deleted (2.1.1). Transconjugants were then compared with regard to their ability to activate the AHL sensor plasmid pAS-C8 via cross-streaking (Fig. 37). *CepI* was not overexpressed in strain H111-rsaM, because the mutant already produces increased

amounts of AHL molecules, thus making it difficult to detect differences in AHL production under the experimental conditions. Interestingly, when plasmid pBBR1MCS5*cepI* was introduced into mutant H111-R (Fig. 37B) and mutant H111-I/R (Fig. 37C) the reporter strain was activated to a greater extent than in mutant H111-I complemented with *cepI* (Fig. 37A). These findings may indicate that RsaM represses the production of AHL molecules by a posttranscriptional mechanism (see 4.1.1, Discussion).

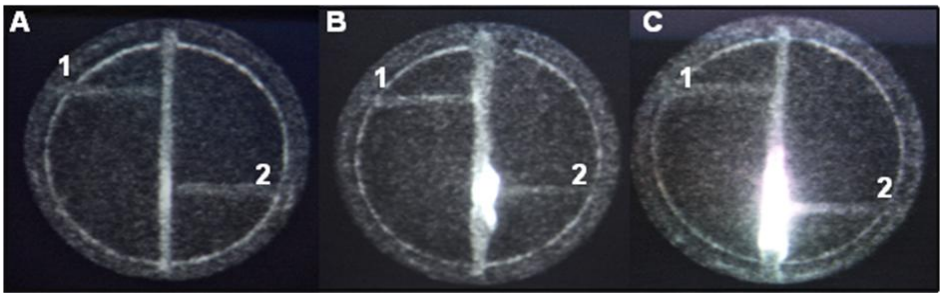


Fig. 37. AHL production of *B. cenocepacia* H111 mutants (1) and *cepI* complemented mutants harbouring plasmid pBBR1MCS*cepI* (2). The H111 *cepI* mutant H111-I (**A**), the *cepR* mutant H111-R (**B**) and mutant H111-I/R, which has a deletion in all three genes *cepR*, *rsaM* and *cepI* (**C**), were streaked against the reporter strain *Pseudomonas putida* F117 (pAS-C8) (2.1.1). The expression of GFP was visualized after an incubation time of 24h at 30°C (2.4.2.2).

4 Discussion and Outlook

4.1. Quorum sensing in *B. cenocepacia* H111

Our understanding of the QS circuitry has expanded with the identification and characterization of the *B. cenocepacia* regulatory protein RsaM and the orphan LuxR homologue CepR2. The current knowledge of the CepIR network of *B. cenocepacia* H111 and the regulatory dependencies observed in this study are summarised and described in the hypothetical model below (Fig. 38). It is important to note, that RsaM and CepR2 seem to function as downstream regulators of the CepIR systems, and might be important for balancing and fine-tuning of the QS network in *B. cenocepacia* H111. It seems remarkable that the deletion of *rsaM* or *cepR2* did only slightly affect the expression of QS-regulated traits in H111 which have been analysed in this study.

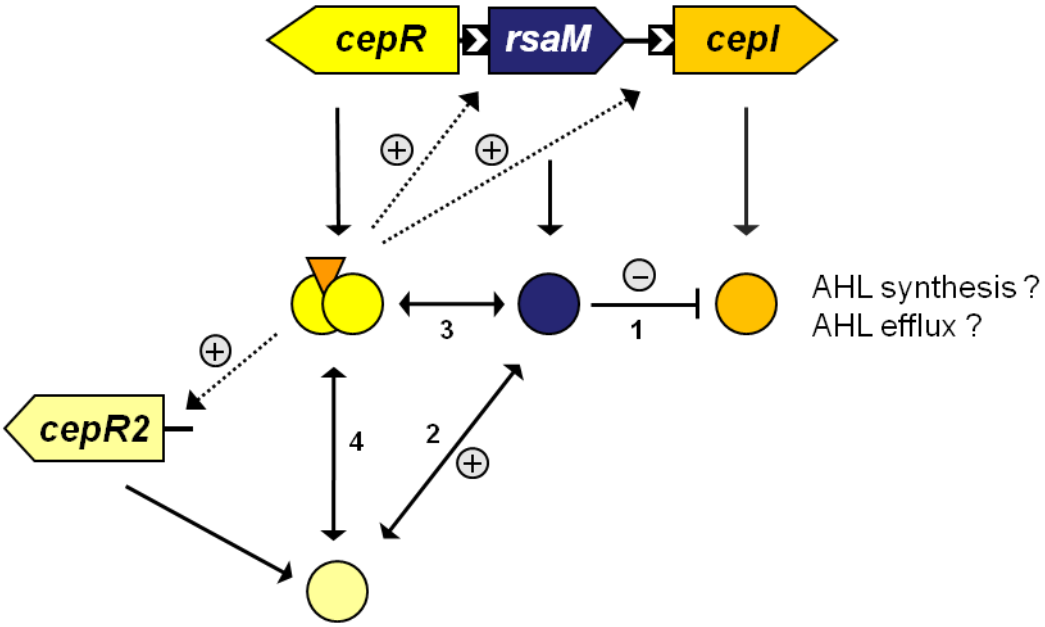


Fig. 38. Hypothetical model of the CepIR QS network in *B. cenocepacia* H111. Shown is the CepIR system which comprises *cepR* encoding the transcriptional regulator CepR, and *cepI* encoding the acyl-homoserine lactone (AHL) synthase CepI. The picture illustrates the genes *rsaM* encoding a protein of unknown function which affects the QS circuitry, and *cepR2* encoding the orphan LuxR homologue CepR2. In complex with AHL molecules, CepR functions as an activator of transcription (dashes lines) of the genes *rsaM*, *cepI* and *cepR2*. The complex then binds to promoter regions containing *cep* boxes (upstream of *rsaM* and *cepI*), and stimulates the expression of *cepR2* through the activation of downstream regulators (indirectly) or by binding to yet unidentified elements within the promoter region of *cepR2*. CepR2 is thought to act as an AHL-independent transcriptional regulator by binding to yet unknown promoter elements which resemble a *lux box* motif. Four regulatory dependencies, possibly acting at a posttranscriptional level, might be proposed on the basis of observations made in this study: RsaM functions as a repressor of CepI activity (AHL synthesis/ efflux) (1), stimulates the activity of the CepR2 protein (2), modulates the activity of CepR (3), and CepR2 and CepR interact with each other and thereby modulate their activities (4).

4.1.1. The RsaM regulator and production of AHL molecules

A recent study reported the identification of two QS systems in the plant pathogen *Pseudomonas fuscovaginae* (Mattiuzzo *et al.*, 2010). One of the QS systems contains a gene, named *rsaM*, encoding a protein of 167 amino acids. This protein was shown to function as a negative regulator of AHL production. In this study, we characterized the RsaM homologue (BCAM1869) of *B. cenocepacia* H111. BCAM1869 is well conserved among Bcc species, albeit the protein lengths can vary considerably, as for instance BCAM1869 (147 amino acids) and Bamb_6054 in *Burkholderia ambifaria* (165 amino acids, Fig. 39).

```
BCAM1869        --------------------------MTSPLLHPVPG----PSPDGYVRLSEGALAALVL
Bamb_6054       MFEGNGRHSRAMRSGRDTRQTRRLLLDLKPDGHAMPAYFDTCSHDGYVRVSATQLASITL
RsaM            MSEDARDSPDSNALEFGYQTCGGIYLRKQNQQSSRAIHMQSLAPDQLVRLSYPELIDLSF
                                          .    . .      : *  **:*   *  : :

BCAM1869        DHVASGLDPSLLAELRDNAIDARLAGYTEWHRTAGAGVAYVTVGWDWYLERATGTFVIAG
Bamb_6054       ALLYAACDERVLSDAWDLGLPACHAGHCEWIDTN--WRPPMTFGWAWLTDRDR-TCRMFP
RsaM            QPYLAWIDTSLTAELKEFGLPVAYAGYSEWECQDS--APKLSISWNWFKEAFSGKVLIAP
                    :  *  : ::  : .: .  **: **        . ::..* *  :    .  :

BCAM1869        GDVRSNVMAIDAKGADIGMLRTAAALAARLAALDWPAAVASALLGHNDAYHAGPTLQ
Bamb_6054       NSVTSNVMIVCARGYDVGPDHTRSLLADWINTLPWTVSVR--------AHLALPVL-
RsaM            GGISCNIMLRSPRGYDLGPDMTQQLLLVWISRQGLECKLP--------AGLMFDRES
                ..: .*:*   .:* *:*   *   *   :        :         *
```

Fig. 39. Amino acid sequence alignment of *B. cenocepacia* RsaM with homologous proteins. Aligned are deduced amino acid sequences of *B. cenocepacia* H111 RsaM (BCAM1869), an RsaM homologue of *B. ambifaria* (Bamb_6054), and the RsaM protein of *Pseudomonas fuscovaginae* (RsaM) with * (identical amino acids), **:** (conserved amino acids), **.** (weakly conserved amino acids).

The close vicinity of the *rsaM* promoter elements to the predicted translational start methionine of BCAM1869 led us to speculate if the start codon of RsaM might rather be an alternative start amino acid, possibly the first leucine in the annotated sequence (Fig. 39). Interestingly, the genes encoding *B. cenocepacia* RsaM or homologues are in all cases located upstream of an AHL synthase-encoding gene, and are also always orientated in the same direction. This observation might indicate that i) the two genes are cotranscribed and that ii) RsaM might be important for the functioning of LuxI-like proteins. RsaM paralogues are found in *Burkholderia ambifaria*, *Burkholderia pseudomallei* and *Burkholderia thailandiensis* but RsaM homologous proteins are missing in *Burkholderia xenovorans* and *Burkholderia phymatum*.

In analogy to the observations made in *P. fuscovaginae*, RsaM has been identified as a repressor of AHL production in *B. cenocepacia* H111 (Fig. 37). Whereas RsaM has been

suggested to control transcription of the AHL synthase PfsI in *P. fuscovaginae* (Mattiuzzo *et al*., 2010), our study indicated that the reduction of AHL levels in *B. cenocepacia* H111 is most likely not due to inhibition of *cepI* transcription by RsaM. In fact, transcription of *cepI* was strongly down-regulated in the *rsaM* mutant (see 4.1.2). Also, the transcriptome of mutant R26 showed reduced levels of *cepI* transcripts. Overexpression of *cepI* under the control of the *lac* promoter (plasmid pBBR1MCS5*cepI*) enhanced the production of AHL molecules in the absence of RsaM. The possibility that RsaM encodes for an AHL lactonase was excluded in this study because i) sequence inspection of the RsaM protein did not reveal any obvious metal binding motifs characteristic of lactonases and ii) the overexpression of *rsaM* did not result in the degradation of AHL molecules in an *E. coli* background. It is important to note that the experiments in this work showed that RsaM negatively affects extracellular levels of AHL molecules in strain H111. As it seems possible that RsaM might affect the efflux of AHL molecules rather than their synthesis it would be revealing to quantify the cellular amounts of AHL molecules in mutant H111-rsaM and *rsaM* complemented strains as it has been described in another study (Chan *et al.*, 2007).

QS systems have been shown to be subject to complex regulation by the aid of various global regulators (Venturi, 2006). A major regulator of AHL production in *P. aeruginosa* is the RsaL protein which represses the transcription of the *P. aeruginosa lasI* and *P. putida ppuI* AHL synthases (Rampioni *et al.*, 2006, Rampioni *et al.*, 2007). Interestingly, *rsaL* is also localized between the genes encoding the LasIR or PpuIR QS systems but, in contrast to *rsaM*, divergently transcribed from the AHL synthase-encoding genes. Notably, RsaL homologues seem to be also encoded in genomes of *B. xenovorans* and *B. phymatum*, which, on the other hand, do not seem to contain a *rsaM* gene. Other *Pseudomonas* regulators which function as QS repressors include the LuxR-like orphan QscR (see 4.1.2), the post-transcriptional regulator RsmA, and the *Pseudomonas putida* Lon protease, which has been demonstrated to affect PpuR protein levels (Bertani *et al.*, 2007). RsmA, the regulator of secondary metabolites, is considered to function as an RNA-binding protein that reduces transcript levels of *expI* in *Erwinia carotovora* (Cui *et al*., 1995) or levels of *rhlI* and *lasI* in *P. aeruginosa* (Pessi *et al.*, 2001). It was shown that RsmA specifically binds to promoter region of target genes at the RBS, thereby affecting their translation rate (Pessi *et al*., 2001, Heeb *et al.*, 2006). It seems possible, that RsmA and RsaM have a similar mode of action. The finding that promoter activities of *cepI* were decreased in the H111 *rsaM* mutant does not seem to correlate with the observation that mutant H111-rsaM exhibited approximately two-fold elevated levels of C6-HSL and C8-HSL. Interestingly, similar regulatory trends were also

observed for the *P. aeruginosa* RsmA regulator. It was found that the mutant produced increased levels of C4-HSL but activities of a translational *rhlI-lacZ* fusion were diminished when *rsmA* was deleted (Pessi *et al*., 2001).

Our studies suggest that RsaM may boost the activity of the orphan LuxR homologue CepR2, which has been identified to control the production of the siderophore pyochelin (see 4.1.2). This hypothesis is mainly based on the observation that pyochelin biosynthesis genes were strongly down-regulated in the transposon insertion mutant R26, but that overexpression of *rsaM* stimulated the CepR2-mediated production of pyochelin. We have suggested a similar regulatory model in which the transcriptional regulator CepR is required for the expression of CepR2, which, in turn, activates the expression of pyochelin biosynthesis genes (Malott *et al*., 2009) (see 4.1.2). The production of pyochelin was clearly reduced when culture samples of mutant H111-R and mutant R26 were spotted on CAS agar. However, the *rsaM* mutant exhibited only a minor decrease in pyochelin production when compared to the wild type under the same assay conditions. These results indicated that i) RsaM may modulate the activity of CepR2 only when it is present at high concentrations or ii) the lack of the CepR regulator in the *rsaM* complemented mutant R26 and mutant H111-R might favour a stimulation of RsaM thus affecting activity of CepR2. The observed stimulation of RsaM on the expression of siderophores does not seem to trigger an overproduction of pyochelin as observed with pyochelin extracts from *cepR2* complemented H111 strains which were grown in liquid cultures. However, an overproduction of pyochelin was also not observed when pyochelin extracts of *cepR* complemented strains were analysed on CAS agar.
The finding that RsaM may interact with QS transcriptional regulators of other bacterial genera, such as LuxR from *Vibrio fischeri*, supports our assumption that RsaM influences the activity of LuxR-like proteins (see 4.1.2). It seems likely that RsaM also governs the activity of the CepR transcriptional regulator, either directly or indirectly, by influencing the activities of CepR2 (Fig. 38, see 4.1.2). This assumption is supported by the finding that mutant H111-rsaM was impaired in the ability to swarm or to form a biofilm, phenoytpes which are positively regulated by CepIR circuitries. However, the observation that RsaM did not influence CepR-mediated activation of the reporter plasmid pAS-C8 in *B. cenocepacia* H111, contradicts the assumption that RsaM modulates the function of CepR.

The orphan LuxR homologue CepR2 has been shown to function as an AHL-independent transcriptional regulator (Malott *et al*., 2009). A regulatory model (Fig. 38), in which RsaM

represses the production of AHL molecules, thereby diminishing the functioning of CepR, and then again stimulates the activity of CepR2, has been proposed. It is important to note that the results obtained in this study do not indicate that CepR2 is involved in the repression of AHL production, as complementation of mutant H111-R2 with *rsaM* diminished the production of AHL signal molecules. The decrease of *cepI* promoter activity in the *rsaM* mutant might, on the other hand, be caused by an altered activity of CepR2 (see 4.1.2).

4.1.2. The orphan LuxR homologue CepR2

CepR2 belongs to the "orphaned" LuxR homologues, since the *cepR2* gene is not accompanied by an adjacent gene encoding a LuxI-like protein. CepR2 is the first orphan LuxR homologue which has been identified in Bcc species (Malott *et al*., 2009). Orphan LuxR regulators have, however, been previously identified in *B. pseudomallei* (Kiratisin & Sanmee, 2008), *B. mallei* (Ulrich *et al*., 2004), and with QscR, the quorum sensing control repressor, in *Pseudomonas aeruginosa* (Chugani *et al.*, 2001). Our study has revealed that CepR2 acts as an AHL-independent transcriptional regulator: the LuxR orphan was shown to induce the transcription of the *Vibrio fischeri luxI* gene in an *E. coli* background, and the expression of *luxI* was not enhanced but also not impaired when AHL molecules were amended to the medium. Against our perceptions, we observed that CepR2 does not seem to activate the promoter of the H111 *cepI* gene. These results are rather surprising, also, since orphan LuxR homologues like BpsR4 and BpsR5 in *B. pseudomallei* (Kiratisin & Sanmee, 2008) or QscR in *P. aeruginosa* (Chugani *et al*., 2001) have been recognized as transcriptional regulators of AHL synthase-encoding genes. However, and in agreement with our findings, we did not detect any obvious differences between the amounts of AHL molecules produced by the H111 *cepR2* mutant and by the wild type strain. CepR2 was moreover seen to stimulate the transcription of *pchR* encoding a transcriptional regulator of pyochelin production, and the transcription of the lectin-encoding operon *bclACB* in *B. cenocepacia* H111. Promoter sequences of these genes did not exhibit motifs similar to that of a *lux* box. Preliminary experiments have indicated that CepR2 seems to specifically bind C8-HSL, C10-HSL, and to a minor degree also 3-oxo-C12-HSL, whereas CepR2 did not seem to interact with C6-HSL (K. Riedel, personal note).

In this study, the *B. cenocepacia* orphan LuxR homologue CepR2 was identified as a positive regulator of pyochelin production in *B. cenocepacia* H111 (Malott *et al*., 2009). While analysing the siderophore production of H111 mutants and complemented strains we

suggested a regulatory cascade in which CepR induces the expression of CepR2 in *B. cenocepacia* (Fig. 38), (Malott *et al*., 2009). In fact, our microarray profiling provides the first data in support of this hypothesis, as *cepR2* transcript levels are significantly reduced (-4.1-fold) in the mutant strain H111-R and likewise increased (4.5-fold) in the *cepR* complemented mutant H111-R (*cepR*$^{+}$). Moreover, transcript levels of pyochelin expression genes are reduced in H111-R and increased in H111-R (*cepR*$^{+}$). Genes comprising the *B. cenocepacia* K56-2 CepR2 regulon have been identified in a transcriptomic profiling of a *cepR2* mutant (Malott *et al*., 2009). The regulon consists mostly of those genes which are located adjacent to the *cepR2* locus, including BCAM0184 - 0186 encoding the BclACB lectins, and genes belonging to three yet uncharacterized transcriptional units (BCAM0191 and BCAM0190, BCAM0192 to BCAM0196, and BCAM0199 to BCAM0202). Interestingly, transcription of most of these genes was highly down-regulated in the transcriptome of the H111 *cepR* mutant, suggesting that the expression of these genes is additionally regulated, likely through stimulation of CepR2. In fact, CepR2 was found to be necessary for the full transcription of the *bclACB* operon in strain H111.

Contrary to our microarray data, transcription of the genes comprising the CepR2 regulon was seen to be repressed by CepR2 in strain K56-2, including BCAM0189 (encoding an AraC family regulatory protein), whose transcription was most increased in the absence of *cepR2* (Malott *et al*., 2009). These differences might be caused by a regulation which is triggered by the in strain H111 absent CciIR QS system, which has been suggested to repress the expression of *cepR2* in *B. cenocepacia* K56-2 (O'Grady *et al*., 2009).

The identification and characterization of CepR2 raises many interesting questions. For instance, the biochemistry of CepR2 is unknown: CepR2 might exist as a homodimer even in the absence of AHL molecules, and it might possibly form heterodimers with the CepR regulator. This complex could activate or repress the transcription of QS-regulated genes. The formation of protein complexes of orphan LuxR homologues with other LuxR-like proteins has been shown for BspR4 of *B. pseudomallei* using a LexA-based bacterial protein interaction assay (Kiratisin & Sanmee, 2008). It might be of interest to perform similar experiments in strain H111 for the further characterization of CepR2, since some results obtained in this study suggest that CepR2 influences the activity of CepR, which might be caused by the formation of CepR2-CepR heterodimers.

Indeed, CepR2 seems to be important for full transcriptional activation of CepR-regulated genes in *B. cenocepacia* H111. However, as CepR2 does not appear to bind to H111 DNA

promoter regions comprising a *cep* box motif it may be assumed that CepR2 interacts with additional factors which are absent in *E. coli*. For that reason it will be interesting to determine if CepR2 influences the transcription of *cepI* in different cell backgrounds of *B. cenocepacia* H111. One possibility might be that certain cellular conditions (for instance low concentration of AHL molecules) favour the formation of a CepR2-CepR heterodimer which then acts as a transcriptional regulator of genes. In has to be kept in mind that the activity of CepR2 seems to be affected by RsaM (Fig. 38). In fact, activities of RsaM and CepR2 might even complement one another. Interestingly, both RsaM and CepR2 have independently been shown to be significantly involved in the regulation of the *Vibrio fischeri* LuxIR system: RsaM was shown to repress the activity of the LuxR protein, whereas CepR2 was found to activate the *luxI* promoter regardless of the presence of absence of AHL molecules. It is currently not know, and might therefore be interesting to determine, if the presence of RsaM influences CepR2-mediated, AHL-independent activation of the *luxI* promoter.

4.2. The BclACB lectins

Despite the large amount of information available on lectin structure and carbohydrate binding specificity, relatively little is known about the biological significances of these proteins. Bacterial lectins are commonly thought to play important roles in processes that require cell adhesion, host recognition or defence mechanisms. Expectedly, genomes of bacteria which act as opportunistic pathogens and which interact and compete with their hosts, often comprise one or even several copies of genes which encode lectins (Sudakevitz *et al*., 2002, Zinger-Yosovich *et al*., 2006, Imberty *et al*., 2005, Imberty & Varrot, 2008). The *B. cenocepacia* lectins are orthologues of the extensively studied *Pseudomonas aeruginosa* fucose-binding lectin PA-IIL, also referred to as LecB (Lameignere *et al*., 2008). All three lectins, BclA (BCAM0186), BclC (BCAM0185), and BclB (BCAM0184) share a C-terminal lectin domain with similarity to that of PA-IIL. Lectin BclA is with 129 amino acids the smallest protein among them. This protein has been intensively analysed with respect of its calcium-dependent dimeric arrangement, its binding preference for mannosides, and its strict specificity for oligomannose-type N-glycan structures that are for instance present on human glycoproteins (Lameignere *et al*., 2008, Lameignere *et al*., 2010).

This study showed that the *B. cenocepacia* lectins are important for the structural development of biofilms, thereby providing information on the role of these proteins *in vivo*. A similar observation was made in a study of *P. aeruginosa* PAO1, where a PA-IIL-deficient

mutant was seen to develop thinner biofilms on glass slides than the wild type (Tielker *et al*., 2005). In full agreement with our findings, PA-IIL was shown to be necessary for the bacterial colonization of surfaces. We observed that the absence of *bclACB* did not affect biofilm formation in a microtitre-dish bases assay after an incubation period of two days. Therefore, it has to be assumed that the interaction between Bcl proteins and carbohydrate complexes excreted by *B. cenocepacia* only occurs under certain cellular or environmental conditions. These conditions appear to prevail when biofilms were grown in flow-cells. Interestingly, transcriptome analysis in *P. aeruginosa* also showed that PA-IIL is up-regulated in growing and developing biofilms (Hentzer *et al.*, 2005). Importantly, lectin expression does not seem to be restricted to a sessile lifestyle of the bacteria as PA-IIL (Tielker *et al*., 2005), and in this study BclB, were also detected in planktonic cells. The glycans or exopolysaccharides (EPS) which are produced by Bcc strains (Cerantola *et al.*, 2000), and which might serve as Bcl carbohydrate ligands within developing biofilms, remain to be identified. An interesting candidate for lectin binding might be the heptasaccharide cepacian (Sist *et al.*, 2003), which has been shown to play a role in the establishment of thick biofilms in *B. cenocepacia* (Cunha *et al.*, 2004), and moreover comprises monosaccharide units of D-mannose that might enable lectin binding. It is likely that not only the production of EPS but also its distribution within the biofilm matrix is triggered by certain factors, therefore significantly affecting lectin binding or the structural development of a biofilm. The formation of holes within the *B. cenocepacia* biofilm matrix, as we have observed it with the lectin mutant H111-*bclACB*, suggests that Bcl function goes beyond that of a mediator of cell adhesion. Instead, the Bcl proteins seem to also exert a stabilizing function in a biofilm by specifically cross-linking bacterial cells to carbohydrate complexes which are present in inner parts of microcolonies.

Our study identified that the transcription of the lectin-encoding operon *bclACB* is induced by the CepIR QS system in *B. cenocepacia* H111. A regulatory role of QS on the expression of lectins has been previously suggested in *P. aeruginosa* for PA-IIL, and more recently for BclA in *B. cenocepacia*. In the former case Winzer and colleagues performed immunoblot analysis and showed that PA-IIL synthesis is induced by the LasIR and the RhlIR QS systems (Winzer *et al.*, 2000). Similarly, transcriptome analyses in *B. cenocepacia* K56-2, which used the same custom microarrays as employed in this study, suggested that *bclA* transcription is positively affected by the CepIR system. Importantly, the inspection of the *bclA* promoter regions did not reveal any obvious *lux* box-like elements as they have been identified within

upstream regions of the PA-IIL-encoding gene (Gilboa-Garber *et al.*, 2000). In fact, several observation made in this study indicate that QS is likely to affect BclACB expression on different levels, and that this regulation might involve the activity of other regulators than CepR. The *B. cenocepacia* orphan LuxR homologue CepR2 for instance (Malott *et al*., 2009), was seen to act as a positive regulator of *bclACB* transcription in this study, even though this regulatory trend was not observed in a Western immunoblot analysis. In contrast to our findings, transcription of *bclA* was seen to be negatively affected by the CepR2 transcriptional regulator in strain K56-2 (Malott *et al*., 2009). In this regard, it is important to note that *B. cenocepacia* H111 does not encode the CciIR QS-system, which is characteristic for *B. cenocepacia* ET12 lineage strains such as K56-2. Both the expression of CepR2 and the expression of BclA have been suggested to be additionally controlled by CciIR networking (O'Grady *et al*., 2009). These circumstances might partly explain the observed strain differences in respect to the transcriptional regulation of the *bclACB* operon. The fact that transcriptional activities of *bclA* were not completely diminished in both *cepR* and *cepR2* mutant backgrounds, moreover suggests that *bclACB* transcription might be additionally controlled by yet unknown factors, which might act independent of QS. For *P. aeruginosa* PA-IIL it was shown that its transcription is also regulated by the alternative sigma factor RpoS (Winzer *et al*., 2000).

Employing immunofluorescence labelling we could show that lectin BclB is associated with the cellular surface of *B. cenocepacia* H111. Through Western immunoblot analyses of planktonic cells we were able to identify lectin BclB in not only whole cell but also in the extracellular protein fraction. The finding that the amounts of BclB were greatly diminished in late-stationary phase cultures of H111 was unexpected and would be of interest for future investigations. Notably, the mechanism by which PA-IIL lectins are translocated across the bacterial cytoplasmic membrane raises questions, since a typical secretion signal seems to be missing in Bcl as well as in PA-IIL (Tielker *et al*., 2005). Genes encoding exported proteins have been successfully identified in *Burkholderia* spp. by mutagenesis experiments employing Tn*5*-based transposons which contain truncated *phoA* gene fusions (Burtnick *et al.*, 2001). This approach relies on the fact that PhoA (alkaline phosphatase) activity in an acid phosphatase (*acpA*)-negative mutant can only be detected, if the truncated *phoA* is fused to a gene that encodes an extracytoplasmatically product. Sine *acpA* has been identified in *Burkholderia* spp. (Burtnick *et al.*, 2001), the generation of a H111 *acpA* mutant should not be a problem. In earlier studies in was suggested that lectin PA-IIL mainly localizes in the bacterial cytoplasm (Gilboa-Garber, 1982), and it was also assumed that cell lysis was

responsible for the release of PA-IIL into the extracellular environment (Wentworth J S, 1991). However, subsequent work provided clear evidence that the protein is localized in the outer-membrane of *P. aeruginosa* (Tielker *et al*., 2005).

While PA-IIL family proteins are present as a single copy in the genome of *P. aeruginosa* (PA-IIL), as well as in *Chromobacterium violaceum* (CV-IIL) and *Ralstonia solanacearum* (RS-IIL) (Sudakevitz *et al*., 2002, Zinger-Yosovich *et al*., 2006), it is surprising that the genomes of Bcc species encode multiple PA-IIL-like proteins, some of which carrying an additional N-terminal domain that is variable in size and sequence. Most recently, lectin BclC was shown to encode a superlectin containing in fact two functional lectin domains: the C-terminal BclA-like domain and an N-terminal tumor necrosis factor-like domain encoding a novel lectin with specificity to fucosylated human blood group epitopes (Sulak *et al*., 2010). Because of the differences with respect to their structures and binding affinities it seems very likely that the three Bcl proteins carry out several functions in *B. cenocepacia*. The performance of more detailed investigations on their biological roles will be a challenging task in the future.

5 References

Andersen, J. B., A. Heydorn, M. Hentzer, L. Eberl, O. Geisenberger, B. B. Christensen, S. Molin & M. Givskov, (2001) gfp-based N-acyl homoserine-lactone sensor systems for detection of bacterial communication. *Appl Environ Microbiol* **67**: 575-585.

Andersen, J. B., C. Sternberg, L. K. Poulsen, S. P. Bjorn, M. Givskov & S. Molin, (1998) New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl Environ Microbiol* **64**: 2240-2246.

Arevalo-Ferro C, H. M., Reil G, Görg A, Kjelleberg S, Givskov M, Riedel & E. L. K, (2003) Identification of quorum-sensing regulated proteins in the opportunistic pathogen Pseudomonas aeruginosa by proteomics. . *Environ Microbiol* **5**: 1350-1369.

Baldwin, A., E. Mahenthiralingam, P. Drevinek, P. Vandamme, J. R. Govan, D. J. Waine, J. J. LiPuma, L. Chiarini, C. Dalmastri, D. A. Henry, D. P. Speert, D. Honeybourne, M. C. Maiden & C. G. Dowson, (2007) Environmental Burkholderia cepacia complex isolates in human infections. *Emerg Infect Dis* **13**: 458-461.

Baldwin, A., P. A. Sokol, J. Parkhill & E. Mahenthiralingam, (2004) The Burkholderia cepacia epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in Burkholderia cenocepacia. *Infect Immun* **72**: 1537-1547.

Bertani, I., G. Rampioni, L. Leoni & V. Venturi, (2007) The Pseudomonas putida Lon protease is involved in N-acyl homoserine lactone quorum sensing regulation. *BMC Microbiol* **7**: 71.

Better, M., B. Lewis, D. Corbin, G. Ditta & D. R. Helinski, (1983) Structural relationships among Rhizobium meliloti symbiotic promoters. *Cell* **35**: 479-485.

Bevivino, A., S. Tabacchioni, L. Chiarini, M. V. Carusi, M. Del Gallo & P. Visca, (1994) Phenotypic comparison between rhizosphere and clinical isolates of Burkholderia cepacia. *Microbiology* **140 (Pt 5)**: 1069-1077.

Birnboim, H. C., (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. *Methods Enzymol* **100**: 243-255.

Bochner, B. R., P. Gadzinski & E. Panomitros, (2001) Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. *Genome Res* **11**: 1246-1255.

Boyer, H. W. & D. Roulland-Dussoix, (1969) A complementation analysis of the restriction and modification of DNA in Escherichia coli. *J Mol Biol* **41**: 459-472.

Bradford, M. M., (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248-254.

Brenner, S., (1974) The Genetics of CAENORHABDITIS ELEGANS. *Genetics* **77**: 71-94.

Burtnick, M., A. Bolton, P. Brett, D. Watanabe & D. Woods, (2001) Identification of the acid phosphatase (acpA) gene homologous in pathogenic and non-pathogenic Burkholderia spp. facilitates TnphoA mutagenesis. *Microbiology* **147**: 111-120.

Carlier, A., L. Burbank & S. B. von Bodman, (2009) Identification and characterization of three novel EsaI/EsaR quorum-sensing controlled stewartan exopolysaccharide biosynthetic genes in Pantoea stewartii ssp. stewartii. *Mol Microbiol* **74**: 903-913.

Cerantola, S., J. Bounery, C. Segonds, N. Marty & H. Montrozier, (2000) Exopolysaccharide production by mucoid and non-mucoid strains of Burkholderia cepacia. *FEMS Microbiol Lett* **185**: 243-246.

Chambers, C. E., E. I. Lutter, M. B. Visser, P. P. Law & P. A. Sokol, (2006) Identification of potential CepR regulated genes using a cep box motif-based search of the Burkholderia cenocepacia genome. *BMC Microbiol* **6**: 104.

Chan, Y. Y., H. S. Bian, T. M. Tan, M. E. Mattmann, G. D. Geske, J. Igarashi, T. Hatano, H. Suga, H. E. Blackwell & K. L. Chua, (2007) Control of quorum sensing by a Burkholderia pseudomallei multidrug efflux pump. *J Bacteriol* **189**: 4320-4324.

Chen, J. S., K. A. Witzmann, T. Spilker, R. J. Fink & J. J. LiPuma, (2001) Endemicity and inter-city spread of Burkholderia cepacia genomovar III in cystic fibrosis. *J Pediatr* **139**: 643-649.

Christensen, B. B., C. Sternberg, J. B. Andersen, R. J. Palmer, Jr., A. T. Nielsen, M. Givskov & S. Molin, (1999) Molecular tools for study of biofilm physiology. *Methods Enzymol* **310**: 20-42.

Chugani, S. A., M. Whiteley, K. M. Lee, D. D'Argenio, C. Manoil & E. P. Greenberg, (2001) QscR, a modulator of quorum-sensing signal synthesis and virulence in Pseudomonas aeruginosa. *Proc Natl Acad Sci U S A* **98**: 2752-2757.

Ciccillo, F., A. Fiore, A. Bevivino, C. Dalmastri, S. Tabacchioni & L. Chiarini, (2002) Effects of two different application methods of Burkholderia ambifaria MCI 7 on plant growth and rhizospheric bacterial diversity. *Environ Microbiol* **4**: 238-245.

Clark, D. J. & O. Maaløe, (1967) DNA replication and the division cycle in Escherichia coli. *Journal of Molecular Biology* **23**: 99-112.

Coenye, T. & J. J. LiPuma, (2002) Multilocus restriction typing: a novel tool for studying global epidemiology of Burkholderia cepacia complex infection in cystic fibrosis. *J Infect Dis* **185**: 1454-1462.

Coenye, T., E. Mahenthiralingam, D. Henry, J. J. LiPuma, S. Laevens, M. Gillis, D. P. Speert & P. Vandamme, (2001) Burkholderia ambifaria sp. nov., a novel member of the Burkholderia cepacia complex including biocontrol and cystic fibrosis-related isolates. *Int J Syst Evol Microbiol* **51**: 1481-1490.

Coenye, T. & P. Vandamme, (2003) Diversity and significance of Burkholderia species occupying diverse ecological niches. *Environ Microbiol* **5**: 719-729.

Coenye, T. & P. Vandamme, (2007) Burkholderia: Molecular Microbiology and Genomics. *Horizon Bioscience*.

Cox, C. D. & R. Graham, (1979) Isolation of an iron-binding compound from Pseudomonas aeruginosa. *J Bacteriol* **137**: 357-364.

Cui, Y., A. Chatterjee, Y. Liu, C. K. Dumenyo & A. K. Chatterjee, (1995) Identification of a global repressor gene, rsmA, of Erwinia carotovora subsp. carotovora that controls extracellular enzymes, N-(3-oxohexanoyl)-L-homoserine lactone, and pathogenicity in soft-rotting Erwinia spp. *J Bacteriol* **177**: 5108-5115.

Cunha, M. V., S. A. Sousa, J. H. Leitao, L. M. Moreira, P. A. Videira & I. Sa-Correia, (2004) Studies on the involvement of the exopolysaccharide produced by cystic fibrosis-associated isolates of the Burkholderia cepacia complex in biofilm formation and in persistence of respiratory infections. *J Clin Microbiol* **42**: 3052-3058.

Datsenko, K. A. & B. L. Wanner, (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc Natl Acad Sci U S A* **97**: 6640-6645.

Davies, D., (2003) Understanding biofilm resistance to antibacterial agents. *Nat Rev Drug Discov* **2**: 114-122.

De Lorenzo, V. & K. N. Timmis, (1994) Analysis and construction of stable phenotypes in gram-negative bacteria with Tn5- and Tn10-derived minitransposons. *Methods Enzymol* **235**: 386-405.

Devine, J. H., G. S. Shadel & T. O. Baldwin, (1989) Identification of the operator of the lux regulon from the Vibrio fischeri strain ATCC7744. *Proc Natl Acad Sci U S A* **86**: 5688-5692.

Eberl, L. & B. Tummler, (2004) Pseudomonas aeruginosa and Burkholderia cepacia in cystic fibrosis: genome evolution, interactions and adaptation. *Int J Med Microbiol* **294**: 123-131.

Figurski, D. H. & D. R. Helinski, (1979) Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. *Proc Natl Acad Sci U S A* **76**: 1648-1652.

Folsom, B. R., P. J. Chapman & P. H. Pritchard, (1990) Phenol and trichloroethylene degradation by Pseudomonas cepacia G4: kinetics and interactions between substrates. *Appl Environ Microbiol* **56**: 1279-1285.

Fuqua, C., S. C. Winans & E. P. Greenberg, (1996) Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. *Annu Rev Microbiol* **50**: 727-751.

Fuqua, W. C., S. C. Winans & E. P. Greenberg, (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* **176**: 269-275.

Geisenberger, O., M. Givskov, K. Riedel, N. Hoiby, B. Tummler & L. Eberl, (2000) Production of N-acyl-L-homoserine lactones by P. aeruginosa isolates from chronic lung infections associated with cystic fibrosis. *FEMS Microbiol Lett* **184**: 273-278.

Gilboa-Garber, N., (1982) Pseudomonas aeruginosa lectins. *Methods Enzymol* **83**: 378-385.

Gilboa-Garber, N., D. J. Katcoff & N. C. Garber, (2000) Identification and characterization of pseudomonas aeruginosa PA-IIL lectin gene and protein compared to PA-IL. *FEMS Immunol Med Microbiol* **29**: 53-57.

Gotschlich, A., B. Huber, O. Geisenberger, A. Togl, A. Steidle, K. Riedel, P. Hill, B. Tummler, P. Vandamme, B. Middleton, M. Camara, P. Williams, A. Hardman & L. Eberl, (2001) Synthesis of multiple N-acylhomoserine lactones is wide-spread among the members of the Burkholderia cepacia complex. *Syst Appl Microbiol* **24**: 1-14.

Hanahan, D., (1983) Studies on transformation of Escherichia coli with plasmids. *J Mol Biol* **166**: 557-580.

Heeb, S., S. A. Kuehne, M. Bycroft, S. Crivii, M. D. Allen, D. Haas, M. Camara & P. Williams, (2006) Functional analysis of the post-transcriptional regulator RsmA reveals a novel RNA-binding site. *J Mol Biol* **355**: 1026-1036.

Hentzer, M., L. Eberl & M. Givskov, (2005) Transcriptome analysis of Pseudomonas aeruginosa biofilm development: anaerobic respiration and iron limitation. *Biofilms* **2**: 37-61.

Heydorn, A., A. T. Nielsen, M. Hentzer, C. Sternberg, M. Givskov, B. K. Ersboll & S. Molin, (2000) Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology* **146 (Pt 10)**: 2395-2407.

Hoang, T. T., R. R. Karkhoff-Schweizer, A. J. Kutchma & H. P. Schweizer, (1998) A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. *Gene* **212**: 77-86.

Holden, M. T., H. M. Seth-Smith, L. C. Crossman, M. Sebaihia, S. D. Bentley, A. M. Cerdeno-Tarraga, N. R. Thomson, N. Bason, M. A. Quail, S. Sharp, I. Cherevach, C. Churcher, I. Goodhead, H. Hauser, N. Holroyd, K. Mungall, P. Scott, D. Walker, B. White, H. Rose, P. Iversen, D. Mil-Homens, E. P. Rocha, A. M. Fialho, A. Baldwin, C. Dowson, B. G. Barrell, J. R. Govan, P. Vandamme, C. A. Hart, E. Mahenthiralingam & J. Parkhill, (2009) The genome of Burkholderia cenocepacia J2315, an epidemic pathogen of cystic fibrosis patients. *J Bacteriol* **191**: 261-277.

Holmes, A., J. Govan & R. Goldstein, (1998) Agricultural use of Burkholderia (Pseudomonas) cepacia: a threat to human health? *Emerg Infect Dis* **4**: 221-227.

Hope, I. A., (1999) *C. elegans : a practical approach*, p. xv, 281 p. Oxford University Press, Oxford.

Huber, B., F. Feldmann, M. Kothe, P. Vandamme, J. Wopperer, K. Riedel & L. Eberl, (2004) Identification of a novel virulence factor in Burkholderia cenocepacia H111 required for efficient slow killing of Caenorhabditis elegans. *Infect Immun* **72**: 7220-7230.

Huber, B., K. Riedel, M. Hentzer, A. Heydorn, A. Gotschlich, M. Givskov, S. Molin & L. Eberl, (2001) The cep quorum-sensing system of Burkholderia cepacia H111 controls biofilm formation and swarming motility. *Microbiology* **147**: 2517-2528.

Huber, B., K. Riedel, M. Kothe, M. Givskov, S. Molin & L. Eberl, (2002) Genetic analysis of functions involved in the late stages of biofilm development in Burkholderia cepacia H111. *Mol Microbiol* **46**: 411-426.

Imberty, A., E. P. Mitchell & M. Wimmerova, (2005) Structural basis of high-affinity glycan recognition by bacterial and fungal lectins. *Curr Opin Struct Biol* **15**: 525-534.

Imberty, A. & A. Varrot, (2008) Microbial recognition of human cell surface glycoconjugates. *Curr Opin Struct Biol* **18**: 567-576.

Isles, A., I. Maclusky, M. Corey, R. Gold, C. Prober, P. Fleming & H. Levison, (1984) Pseudomonas cepacia infection in cystic fibrosis: an emerging problem. *J Pediatr* **104**: 206-210.

Jan Molin, S. M., (2000) Complex Adaptive Systems Ecology. *Technical University of Denmark*.

Jander, G., L. G. Rahme & F. M. Ausubel, (2000) Positive correlation between virulence of Pseudomonas aeruginosa mutants in mice and insects. *J Bacteriol* **182**: 3843-3845.

Jimenez, L., (2001) Molecular diagnosis of microbial contamination in cosmetic and pharmaceutical products: a review. *J AOAC Int* **84**: 671-675.

Johnson, D. A., S. G. Tetu, K. Phillippy, J. Chen, Q. Ren & I. T. Paulsen, (2008) High-throughput phenotypic characterization of Pseudomonas aeruginosa membrane transport genes. *PLoS Genet* **4**: e1000211.

Jones, A. L., T. J. Beveridge & D. E. Woods, (1996) Intracellular survival of Burkholderia pseudomallei. *Infect Immun* **64**: 782-790.

Kiratisin, P. & S. Sanmee, (2008) Roles and interactions of Burkholderia pseudomallei BpsIR quorum-sensing system determinants. *J Bacteriol* **190**: 7291-7297.

Kooi, C., B. Subsin, R. Chen, B. Pohorelic & P. A. Sokol, (2006) Burkholderia cenocepacia ZmpB is a broad-specificity zinc metalloprotease involved in virulence. *Infect Immun* **74**: 4083-4093.

Köthe, M., M. Antl, B. Huber, K. Stoecker, D. Ebrecht, I. Steinmetz & L. Eberl, (2003) Killing of Caenorhabditis elegans by Burkholderia cepacia is controlled by the cep quorum-sensing system. *Cell Microbiol* **5**: 343-351.

Kovach, M. E., P. H. Elzer, D. S. Hill, G. T. Robertson, M. A. Farris, R. M. Roop, 2nd & K. M. Peterson, (1995) Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**: 175-176.

Kovach, M. E., R. W. Phillips, P. H. Elzer, R. M. Roop, 2nd & K. M. Peterson, (1994) pBBR1MCS: a broad-host-range cloning vector. *Biotechniques* **16**: 800-802.

Laemmli, U. K., (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.

Lameignere, E., L. Malinovska, M. Slavikova, E. Duchaud, E. P. Mitchell, A. Varrot, O. Sedo, A. Imberty & M. Wimmerova, (2008) Structural basis for mannose recognition by a lectin from opportunistic bacteria Burkholderia cenocepacia. *Biochem J* **411**: 307-318.

Lameignere, E., T. C. , R. R. Shiao, M. Wimmerova, F. Dubreuil & A. V. A. Imberty, (2010) Structural basis of the affinity for oligomannosides and analogs displayed by BC2L-A, a Burkholderia cenocepacia soluble lectin. *Glycobiology* **20**: 87-98.

Lessie, T. G., W. Hendrickson, B. D. Manning & R. Devereux, (1996) Genomic complexity and plasticity of Burkholderia cepacia. *FEMS Microbiol Lett* **144**: 117-128.

Lewenza, S., B. Conway, E. P. Greenberg & P. A. Sokol, (1999) Quorum sensing in Burkholderia cepacia: identification of the LuxRI homologs CepRI. *J Bacteriol* **181**: 748-756.

Lewenza, S. & P. A. Sokol, (2001) Regulation of ornibactin biosynthesis and N-acyl-L-homoserine lactone production by CepR in Burkholderia cepacia. *J Bacteriol* **183**: 2212-2218.

Lindhardt TJ, B. R., Danies L, Mayerl R, Pickenhagen W, (1989) Microbially produced rhamnolipid as a source of rhamnose. *Biotechnol. Bioeng.* **33**: 365-368.

LiPuma, J. J., J. E. Mortensen, S. E. Dasen, T. D. Edlind, D. V. Schidlow, J. L. Burns & T. L. Stull, (1988) Ribotype analysis of Pseudomonas cepacia from cystic fibrosis treatment centers. *J Pediatr* **113**: 859-862.

Ma, L., M. Conover, H. Lu, M. R. Parsek, K. Bayles & D. J. Wozniak, (2009) Assembly and development of the Pseudomonas aeruginosa biofilm matrix. *PLoS Pathog* **5**: e1000354.

Mahenthiralingam, E., A. Baldwin & C. G. Dowson, (2008) Burkholderia cepacia complex bacteria: opportunistic pathogens with important natural biology. *J Appl Microbiol* **104**: 1539-1551.

Mahenthiralingam, E., A. Baldwin & P. Vandamme, (2002) Burkholderia cepacia complex infection in patients with cystic fibrosis. *J Med Microbiol* **51**: 533-538.

Mahenthiralingam, E., T. Coenye, J. W. Chung, D. P. Speert, J. R. Govan, P. Taylor & P. Vandamme, (2000) Diagnostically and experimentally useful panel of strains from the Burkholderia cepacia complex. *J Clin Microbiol* **38**: 910-913.

Mahenthiralingam, E., T. A. Urban & J. B. Goldberg, (2005) The multifarious, multireplicon Burkholderia cepacia complex. *Nat Rev Microbiol* **3**: 144-156.

Mahenthiralingam, E., P. Vandamme, M. E. Campbell, D. A. Henry, A. M. Gravelle, L. T. Wong, A. G. Davidson, P. G. Wilcox, B. Nakielna & D. P. Speert, (2001) Infection with Burkholderia cepacia complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace Burkholderia multivorans. *Clin Infect Dis* **33**: 1469-1475.

Malott, R. J., A. Baldwin, E. Mahenthiralingam & P. A. Sokol, (2005) Characterization of the cciIR quorum-sensing system in Burkholderia cenocepacia. *Infect Immun* **73**: 4982-4992.

Malott, R. J., E. P. O'Grady, J. Toller, S. Inhulsen, L. Eberl & P. A. Sokol, (2009) A Burkholderia cenocepacia orphan LuxR homolog is involved in quorum-sensing regulation. *J Bacteriol* **191**: 2447-2460.

Mattiuzzo, M., I. Bertani, S. Ferluga, L. Cabrio, J. Bigirimana, C. Guarnaccia, S. Pongor, H. Maraite & V. Venturi, (2010) The plant pathogen Pseudomonas fuscovaginae contains two conserved quorum sensing systems involved in virulence and negatively regulated by RsaL and the novel regulator RsaM. *Environ Microbiol*.

McClean, K. H., M. K. Winson, L. Fish, A. Taylor, S. R. Chhabra, M. Camara, M. Daykin, J. H. Lamb, S. Swift, B. W. Bycroft, G. S. Stewart & P. Williams, (1997) Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology* **143 (Pt 12)**: 3703-3711.

Meselson, M. & R. Yuan, (1968) DNA restriction enzyme from E. coli. *Nature* **217**: 1110-1114.

Meyer, J. M., and M. A. Abdallah, (1978) The fluorescent pigment of Pseudomonas fluorescens: biosyntehsis, purification and physico-chemical properties. *J. Gen. Microbiol* **107**: 319-328.

Miller, J., (1972) *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Miller, S. C., J. J. LiPuma & J. L. Parke, (2002) Culture-based and non-growth-dependent detection of the Burkholderia cepacia complex in soil environments. *Appl Environ Microbiol* **68**: 3750-3758.

Miller, V. L. & J. J. Mekalanos, (1988) A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in Vibrio cholerae requires toxR. *J Bacteriol* **170**: 2575-2583.

Nealson, K. H., T. Platt & J. W. Hastings, (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. *J Bacteriol* **104**: 313-322.

O'Grady, E. P., D. F. Viteri, R. J. Malott & P. A. Sokol, (2009) Reciprocal regulation by the CepIR and CciIR quorum sensing systems in Burkholderia cenocepacia. *BMC Genomics* **10**: 441.

O'Toole, G. A. & R. Kolter, (1998) Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol Microbiol* **28**: 449-461.

Parke, J. L. & D. Gurian-Sherman, (2001) Diversity of the Burkholderia cepacia complex and implications for risk assessment of biological control strains. *Annu Rev Phytopathol* **39**: 225-258.

Parke, J. L., R.E. Rand, A.E. Joy, E.B. and King, (1991) Biological control of Pythium damping-off and Aphanomyces root rot of peas by application of Pseudomonas cepacia or P. fluorescens to seed. *Plant Dis* **75**: 987-992.

Perkins, D. N., D. J. Pappin, D. M. Creasy & J. S. Cottrell, (1999) Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **20**: 3551-3567.

Pessi, G., F. Williams, Z. Hindle, K. Heurlier, M. T. Holden, M. Camara, D. Haas & P. Williams, (2001) The global posttranscriptional regulator RsmA modulates production of virulence determinants and N-acylhomoserine lactones in Pseudomonas aeruginosa. *J Bacteriol* **183**: 6676-6683.

Rampioni, G., I. Bertani, E. Zennaro, F. Polticelli, V. Venturi & L. Leoni, (2006) The quorum-sensing negative regulator RsaL of Pseudomonas aeruginosa binds to the lasI promoter. *J Bacteriol* **188**: 815-819.

Rampioni, G., F. Polticelli, I. Bertani, K. Righetti, V. Venturi, E. Zennaro & L. Leoni, (2007) The Pseudomonas quorum-sensing regulator RsaL belongs to the tetrahelical superclass of H-T-H proteins. *J Bacteriol* **189**: 1922-1930.

Ratjen, F. & G. Doring, (2003) Cystic fibrosis. *Lancet* **361**: 681-689.

Riedel, K., C. Arevalo-Ferro, G. Reil, A. Gorg, F. Lottspeich & L. Eberl, (2003) Analysis of the quorum-sensing regulon of the opportunistic pathogen Burkholderia cepacia H111 by proteomics. *Electrophoresis* **24**: 740-750.

Riedel, K., M. Hentzer, O. Geisenberger, B. Huber, A. Steidle, H. Wu, N. Hoiby, M. Givskov, S. Molin & L. Eberl, (2001) N-acylhomoserine-lactone-mediated communication between Pseudomonas aeruginosa and Burkholderia cepacia in mixed biofilms. *Microbiology* **147**: 3249-3262.

Romling, U., B. Fiedler, J. Bosshammer, D. Grothues, J. Greipel, H. von der Hardt & B. Tummler, (1994) Epidemiology of chronic Pseudomonas aeruginosa infections in cystic fibrosis. *J Infect Dis* **170**: 1616-1621.

Ryan, R. P., Y. McCarthy, S. A. Watt, K. Niehaus & J. M. Dow, (2009) Intraspecies signaling involving the diffusible signal factor BDSF (cis-2-dodecenoic acid) influences virulence in Burkholderia cenocepacia. *J Bacteriol* **191**: 5013-5019.

Saiman, L., G. Cacalano & A. Prince, (1990) Pseudomonas cepacia adherence to respiratory epithelial cells is enhanced by Pseudomonas aeruginosa. *Infect Immun* **58**: 2578-2584.

Saiman, L. & J. Siegel, (2004) Infection control in cystic fibrosis. *Clin Microbiol Rev* **17**: 57-71.

Sambrook, J., Fritsch, E. F. & T. Maniatis (1989) Molecular Cloning: a Laboratory Manual, 2nd edn. . *Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.*

Schwyn, B., and J. B. Neilands, (1987) Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **160**: 47-56.

Seed, K. D. & J. J. Dennis, (2008) Development of Galleria mellonella as an alternative infection model for the Burkholderia cepacia complex. *Infect Immun* **76**: 1267-1275.

Sist, P., P. Cescutti, S. Skerlavaj, R. Urbani, J. H. Leitao, I. Sa-Correia & R. Rizzo, (2003) Macromolecular and solution properties of Cepacian: the exopolysaccharide produced by a strain of Burkholderia cepacia isolated from a cystic fibrosis patient. *Carbohydr Res* **338**: 1861-1867.

Sokol, P. A., (1986) Production and utilization of pyochelin by clinical isolates of Pseudomonas cepacia. *J Clin Microbiol* **23**: 560-562.

Sokol, P. A., U. Sajjan, M. B. Visser, S. Gingues, J. Forstner & C. Kooi, (2003) The CepIR quorum-sensing system contributes to the virulence of Burkholderia cenocepacia respiratory infections. *Microbiology* **149**: 3649-3658.

Southern, E. M., (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* **98**: 503-517.

Speert, D. P., (2001) Understanding Burkholderia cepacia: epidemiology, genomovars, and virulence. *Infect. Med.* **18**: 49–56.

Stanier, R. Y., N. J. Palleroni & M. Doudoroff, (1966) The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**: 159-271.

Steidle, A., K. Sigl, R. Schuhegger, A. Ihring, M. Schmid, S. Gantner, M. Stoffels, K. Riedel, M. Givskov, A. Hartmann, C. Langebartels & L. Eberl, (2001) Visualization of N-acylhomoserine lactone-mediated cell-cell communication between bacteria colonizing the tomato rhizosphere. *Appl Environ Microbiol* **67**: 5761-5770.

Subsin, B., C. E. Chambers, M. B. Visser & P. A. Sokol, (2007) Identification of genes regulated by the cepIR quorum-sensing system in Burkholderia cenocepacia by high-throughput screening of a random promoter library. *J Bacteriol* **189**: 968-979.

Sudakevitz, D., A. Imberty & N. Gilboa-Garber, (2002) Production, properties and specificity of a new bacterial L-fucose- and D-arabinose-binding lectin of the plant aggressive pathogen Ralstonia solanacearum, and its comparison to related plant and microbial lectins. *J Biochem* **132**: 353-358.

Sulak, O., G. Cioci, M. Delia, M. Lahmann, A. Varrot & A. I. M. Wimmerova, (2010) A TNF-like trimeric lectin domain from Burkholderia cenocepacia with specificity for fucosylated human histo-blood group antigens. *Structure* **18**: 59-72.

Tabacchioni, S., P. Visca, L. Chiarini, A. Bevivino, C. Di Serio, S. Fancelli & R. Fani, (1995) Molecular characterization of rhizosphere and clinical isolates of Burkholderia cepacia. *Res Microbiol* **146**: 531-542.

Tan, M. W., S. Mahajan-Miklos & F. M. Ausubel, (1999) Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis. *Proc Natl Acad Sci U S A* **96**: 715-720.

Tielker, D., S. Hacker, R. Loris, M. Strathmann, J. Wingender, S. Wilhelm, F. Rosenau & K. E. Jaeger, (2005) Pseudomonas aeruginosa lectin LecB is located in the outer membrane and is involved in biofilm formation. *Microbiology* **151**: 1313-1323.

Toller, J., (2008) Untersuchungen zur Funktion von CepR2 im "Quorum sensing" von *Burkholderia cenocepacia*. *Master Thesis, Universität Zürich*.

Tran Van, V., O. Berge, S. Ngo Ke, J. Balandreau, and T. Heulin (2000) Repeated beneficial effects of rice inoculation with a strain of Burkholderia vietnamiensis on early and late yield components in low fertility sulphate acid soils of Vietnam. *Plant Soil* **218**: 273-284.

Tummler, B. & C. Kiewitz, (1999) Cystic fibrosis: an inherited susceptibility to bacterial respiratory infections. *Mol Med Today* **5**: 351-358.

Uehlinger, S., (2009) Identification of Virulence Factors in Burkholderia sp. Using Diverse Infection Hosts. *Dissertation, University of Zurich*.

Uehlinger, S., S. Schwager, S. P. Bernier, K. Riedel, D. T. Nguyen, P. A. Sokol & L. Eberl, (2009) Identification of specific and universal virulence factors in Burkholderia cenocepacia strains by using multiple infection hosts. *Infect Immun* **77**: 4102-4110.

Ulrich, R. L., D. Deshazer, H. B. Hines & J. A. Jeddeloh, (2004) Quorum sensing: a transcriptional regulatory system involved in the pathogenicity of Burkholderia mallei. *Infect Immun* **72**: 6589-6596.

Van Oevelen, S., R. De Wachter, P. Vandamme, E. Robbrecht & E. Prinsen, (2002) Identification of the bacterial endosymbionts in leaf galls of Psychotria (Rubiaceae, angiosperms) and proposal of 'Candidatus Burkholderia kirkii' sp. nov. *Int J Syst Evol Microbiol* **52**: 2023-2027.

Vandamme, P., B. Holmes, T. Coenye, J. Goris, E. Mahenthiralingam, J. J. LiPuma & J. R. Govan, (2003) Burkholderia cenocepacia sp. nov.--a new twist to an old story. *Res Microbiol* **154**: 91-96.

Vandamme, P., B. Holmes, M. Vancanneyt, T. Coenye, B. Hoste, R. Coopman, H. Revets, S. Lauwers, M. Gillis, K. Kersters & J. R. Govan, (1997) Occurrence of multiple genomovars of Burkholderia cepacia in cystic fibrosis patients and proposal of Burkholderia multivorans sp. nov. *Int J Syst Bacteriol* **47**: 1188-1200.

Vanlaere, E., A. Baldwin, D. Gevers, D. Henry, E. De Brandt, J. J. LiPuma, E. Mahenthiralingam, D. P. Speert, C. Dowson & P. Vandamme, (2009) Taxon K, a complex within the Burkholderia cepacia complex, comprises at least two novel species, Burkholderia contaminans sp. nov. and Burkholderia lata sp. nov. *Int J Syst Evol Microbiol* **59**: 102-111.

Vanlaere, E., A. Baldwin, D. Gevers, D. A. Henry, J. J. Lipuma, E. Mahenthiralingam, D. Speert, C. Dowson & P. Vandamme, (2008a) Taxon K, a complex within the *Burkholderia cepacia* complex comprises at least two novel species: *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. . *Int J Syst Evol Microbiol*.

Vanlaere, E., J. J. Lipuma, A. Baldwin, D. Henry, E. De Brandt, E. Mahenthiralingam, D. Speert, C. Dowson & P. Vandamme, (2008b) Burkholderia latens sp. nov., Burkholderia diffusa sp. nov., Burkholderia arboris sp. nov., Burkholderia seminalis sp. nov. and Burkholderia metallica sp. nov., novel species within the Burkholderia cepacia complex. *Int J Syst Evol Microbiol* **58**: 1580-1590.

Venturi, V., (2006) Regulation of quorum sensing in Pseudomonas. *FEMS Microbiol Rev* **30**: 274-291.

Vermis, K., T. Coenye, J. J. LiPuma, E. Mahenthiralingam, H. J. Nelis & P. Vandamme, (2004) Proposal to accommodate Burkholderia cepacia genomovar VI as Burkholderia dolosa sp. nov. *Int J Syst Evol Microbiol* **54**: 689-691.

Visca, P., G. Colotti, L. Serino, D. Verzili, N. Orsi & E. Chiancone, (1992) Metal regulation of siderophore synthesis in Pseudomonas aeruginosa and functional effects of siderophore-metal complexes. *Appl Environ Microbiol* **58**: 2886-2893.

Webb, J. S., L. S. Thompson, S. James, T. Charlton, T. Tolker-Nielsen, B. Koch, M. Givskov & S. Kjelleberg, (2003) Cell death in Pseudomonas aeruginosa biofilm development. *J Bacteriol* **185**: 4585-4592.

Weiner MP, A. C., Jerpseth B, Wells S, Johnson-Browne B, Vaillancourt P (1994) Studier pET system vectors and hosts. *Strateg Mol Biol* **7**: 41–43

Weingart, C. L., C. E. White, S. Liu, Y. Chai, H. Cho, C. S. Tsai, Y. Wei, N. R. Delay, M. R. Gronquist, A. Eberhard & S. C. Winans, (2005) Direct binding of the quorum sensing regulator CepR of Burkholderia cenocepacia to two target promoters in vitro. *Mol Microbiol* **57**: 452-467.

Wentworth J S, A. F. E., Garber N C, Gilboa-Garber N, Paterson C A, Doyle R J. , (1991) Cytoplasmic lectins contribute to the adhesion of P. aeruginosa. *Biofouling* **4**: 99–104.

Whitehead, N. A., A. M. Barnard, H. Slater, N. J. Simpson & G. P. Salmond, (2001) Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol Rev* **25**: 365-404.

Williams, P., K. Winzer, W. C. Chan & M. Camara, (2007) Look who's talking: communication and quorum sensing in the bacterial world. *Philos Trans R Soc Lond B Biol Sci* **362**: 1119-1134.

Winson, M. K., S. Swift, L. Fish, J. P. Throup, F. Jorgensen, S. R. Chhabra, B. W. Bycroft, P. Williams & G. S. Stewart, (1998) Construction and analysis of luxCDABE-based plasmid sensors for investigating N-acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol Lett* **163**: 185-192.

Winsor, G. L., B. Khaira, T. Van Rossum, R. Lo, M. D. Whiteside & F. S. Brinkman, (2008) The Burkholderia Genome Database: facilitating flexible queries and comparative analyses. *Bioinformatics* **24**: 2803-2804.

Winzer, K., C. Falconer, N. C. Garber, S. P. Diggle, M. Camara & P. Williams, (2000) The Pseudomonas aeruginosa lectins PA-IL and PA-IIL are controlled by quorum sensing and by RpoS. *J Bacteriol* **182**: 6401-6411.

Wopperer, J., S. T. Cardona, B. Huber, C. A. Jacobi, M. A. Valvano & L. Eberl, (2006) A quorum-quenching approach to investigate the conservation of quorum-sensing-regulated functions within the Burkholderia cepacia complex. *Appl Environ Microbiol* **72**: 1579-1587.

Wopperer, J. D., (2008) Quorum Sensing and Virulence in Members of the Genus *Burkholderia*. *Dissertation, University of Zurich*.

Zinger-Yosovich, K., D. Sudakevitz, A. Imberty, N. C. Garber & N. Gilboa-Garber, (2006) Production and properties of the native Chromobacterium violaceum fucose-binding lectin (CV-IIL) compared to homologous lectins of Pseudomonas aeruginosa (PA-IIL) and Ralstonia solanacearum (RS-IIL). *Microbiology* **152**: 457-463.

6 Appendix

Figure A1

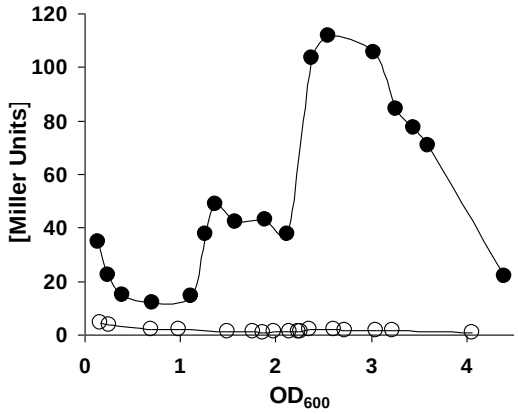
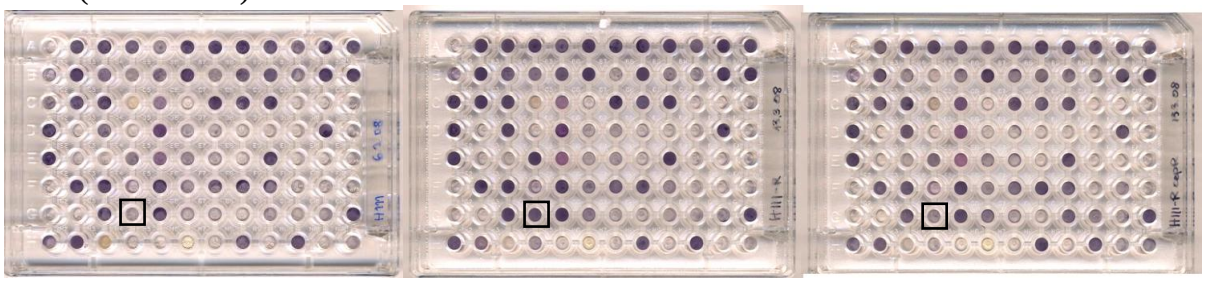


Fig. A1. Transcription of *aidA* is stringently regulated by CepR. The diagram shows a representative example of the promoter activities of *aidA* determined in the *B. cenocepacia* H111 parent (filled circles), and in the *cepR* mutant H111-R (open circels). Transcription of *aidA* (BAS0293) is maximal at an optical density (OD_{600}) of 2.5. ß-galactosidase activities of promoter fusion P_{aidA1}-*lacZ* (2.2.8) were determined in LB-medium throughout the growth curve as measured by absorbance at 600 nm.

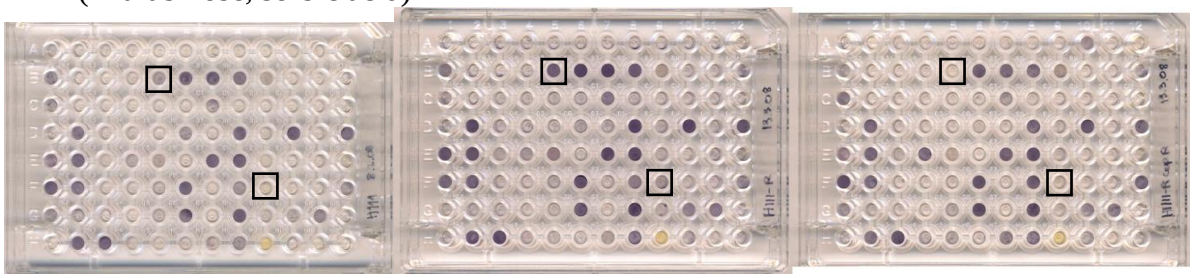
Figure A2

H111 wt **H111-R** **H111-R (*cepR*$^+$)**

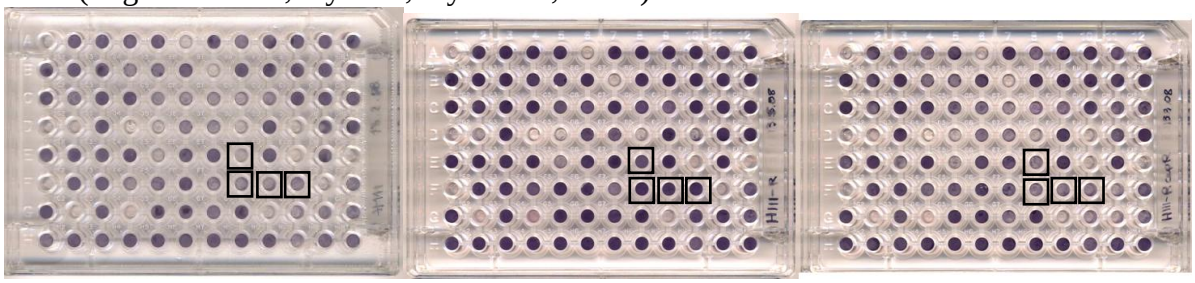
PM1 (L-threonine)



PM2 (D-arabinose, sorbic acid)



PM3 (D-glucosamine, thymine, thymidine, uracil)



PM4 (Methylen diphosphonic acid, p-amino-benzene sulfonic acid)

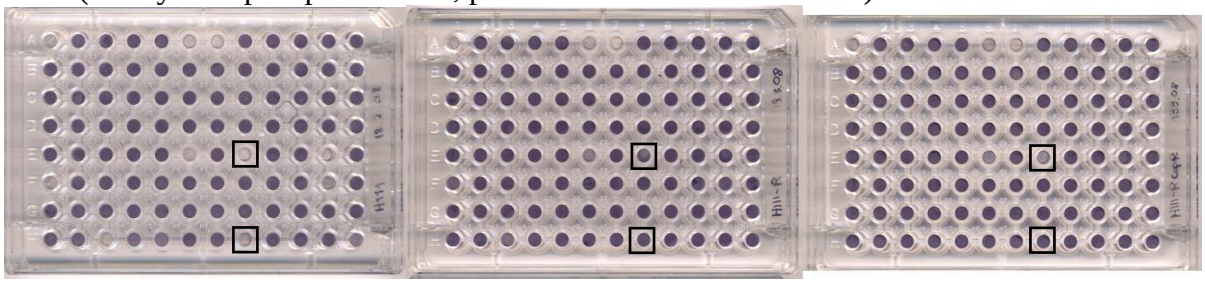


Fig. A2. Metabolic profiling of *B. cenocepacia* H111 strains using BIOLOG microtiter plates. The H111 wild type (wt), mutant H111-R, and the complemented mutant H111-R (*cepR*$^+$) were grown in plates containing minimal medium supplemented with varying carbon (PM1), nitrogen (PM2), sulfur (PM3) and phosphorus sources (PM4). Pictures were taken after an incubation period for 24h at 37°C (2.4.7).

Figure A3

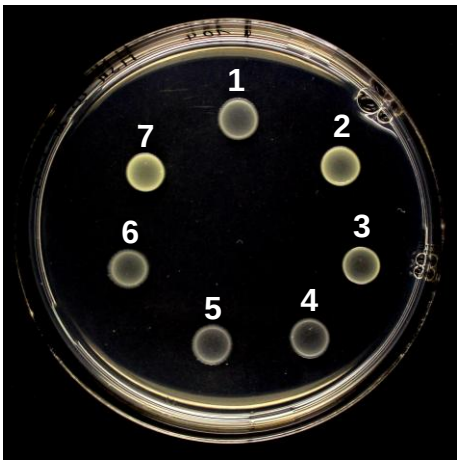


Fig. A3. Growth differences between the *B. cenocepacia* H111 wild type and mutant strains on minimal medium supplemented mit D-arabinose. The *B. cenocepacia* H111 wild type (1), mutant H111-R (2), mutant H111-I (3), *cepR* complemented mutants H111-R (*cepR*$^{+}$) (4) and H111 wt (*cepR*$^{+}$) (5), mutant H111-R2 (6), and H111-R/R2 (7) were grown for 48h at 37°C on mineral salt medium (2.2.1) agar plates supplemented with D-arabinose [10 mM]. Strains were dropped from overnight cultures grown in MSM medium with 10 mM D-arabinose.

Table A1. Genes and ORFs, tRNA and intergenetic regions which are more than 2-fold differentially regulated in the H111-R transcriptome versus the H111 wild type. [a)] : Genes in bold type illustrate QS-regulated genes also identified in the transcriptome analysis of *B. cenocepacia* K56-2 (O'Grady *et al*., 2009) and [b)] : QS-regulated genes identified by alternative approaches. References are: **1**: (Huber *et al*., 2001), **2**: (Riedel *et al*., 2003), **3**: (Huber *et al*., 2004), **4**: (Weingart *et al*., 2005) **5**: (Chambers *et al*., 2006), **6**: (Subsin *et al*., 2007), **7**: (Malott *et al*., 2009) and **8**: (Ryan *et al*., 2009).

Gene name [a)]	Fold change	Description	Reference [b)]
BCAS0293	167,20	nematocidal protein AidA	**2;3;4;5;6;7**
BCAS0292	138,10	conserved hypothetical protein	
BCAM0194	52,24	conserved hypothetical protein	**7**
BCAM0193	47,68	conserved hypothetical protein	**7**
BCAM0192	46,78	conserved hypothetical protein	**7**
BCAM0196	37,72	conserved hypothetical protein	**7**
BCAM0195	34,03	putative non-ribosomal peptide synthetase	**7**
BCAM1871	32,53	conserved hypothetical protein	
BCAM1870	26,99	N-acylhomoserine lactone synthase CepI	**1;4;5**
BCAL3285	25,80	flavohemoprotein	
BCAM0190	22,11	putative aminotransferase - class III	**7**
BCAM0191	12,83	putative non-ribosomal peptide synthetase	**7**
BCAM0200	9,24	efflux system transport protein	**7**
BCAM0186	9,02	lectin	**7**
BCAM2307	8,72	zinc metalloprotease ZmpB	**2;7**
BCAL1677	8,72	putative type-1 fimbrial protein	**2**
BCAL1681	8,57	putative exported protein	
BCAL1678	6,44	putative membrane usher protein precursor	
BCAL1680	6,20	putative type-1 fimbrial protein	
BCAM1869	6,07	conserved hypothetical protein	
BCAL0510	5,67	conserved hypothetical protein	
BCAL1679	5,48	putative fimbrial chaperone	
BCAS0236	5,39	putative haemagglutinin-rel. autotransporter	
BCAM2308	5,32	putative leucyl aminopeptidase precursor	
BCAL1813	5,29	multidrug efflux system outer membrane protein	
BCAM2142	5,28	transport system outer membrane protein	
BCAM0028	5,10	putative FHA-domain protein	
BCAM0184	5,07	lectin	
BCAM2143	5,06	cable pilus associated adhesin protein	
BCAL3178	4,97	LysR family regulatory protein	
BCAS0498	4,94	muconate cycloisomerase I 2	
BCAL0358	4,69	metallo peptidase, family M1	**5**
BCAM2141	4,42	ABC transporter ATP-binding membrane protein	
BCAM2720	4,42	putative phospholipase C	
BCAS0409	4,36	zinc metalloprotease ZmpA	**5;6;7**
BCAM0835	4,32	AraC family regulatory protein	
BCAM0188	4,13	N-acylhomoserine lactone dependent regulatory	**7**
BCAM0393	4,05	putative beta-lactamase, class D	
BCAM0189	3,89	AraC family regulatory protein	**7**
BCAM0392	3,83	putative acetyltransferase	**5**
BCAL1921	3,49	conserved hypothetical protein	
BCAL3179	3,38	probable D-lactate dehydrogenase	
BCAL0121	3,30	aquaporin Z	
BCAM0185	3,25	lectin	**2**
BCAL0831	3,23	putative storage protein	**5**
BCAM0030	3,14	conserved hypothetical protein	
BCAM2140	3,10	transporter system transport protein	
BCAL0833	3,02	putative Acetoacetyl-CoA reductase	
BCAM2684	2,95	putative acetyltransferase	
BCAL1142	2,94	putative transposase	
BCAM2419	2,90	putative outer membrane protein A precursor	
BCAM0166	2,89	NADH dehydrogenase	
BCAL0625	2,81	LysR family regulatory protein	
BCAL2786	2,80	putative selenium-binding protein	
BCAL1731	2,80	Major Facilitator Superfamily protein	
BCAS0259	2,79	putative sodium:dicarboxylate symporter family	
BCAM0622	2,75	two-component regulatory system, sensor kinase	
BCAS0258	2,74	GntR family regulatory protein	
BCAM0130	2,72	putative acyl-CoA dehydrogenase	**6**
BCAM2771	2,68	putative dihydrodipicolinate synthetase	
BCAM0341	2,67	acyl carrier protein phosphodiesterase	**6**
BCAM2340	2,66	putative (R)-3-hydroxydecanoyl-ACP:CoA	
BCAM1697	2,65	putative membrane-associated amino terminal	
BCAM1413a	2,63	conserved hypothetical protein	
BCAM0031	2,55	conserved hypothetical protein	
BCAL3006	2,55	cold shock-like protein	**6**
BCAM2444	2,52	putative exported protein	
BCAL0834	2,48	putative membrane protein	
BCAL0665	2,43	dethiobiotin synthetase	
BCAM1811	2,42	conserved hypothetical protein	
BCAM0633	2,42	conserved hypothetical protein	
BCAS0225	2,39	shiny variant regulator ShvR	
BCAS0497	2,39	catechol 1,2-dioxygenase 2	
BCAL2397	2,38	putative lipoprotein	
BCAM1673	2,38	Major Facilitator Superfamily protein	
BCAM1412	2,38	conserved hypothetical protein	
BCAM1010	2,36	putative UTP-glucose-1-phosphate	
BCAS0335	2,33	putative haemagglutinin-related autotransporter	
BCAM0634	2,31	hypothetical protein	
BCAM1876A	2,30	hypothetical phage protein	
BCAS0249	2,30	chromate resistance transport protein	
BCAM2770	2,23	putative sulfur-binding protein	
BCAL2353	2,23	putative sulfate transporter	
BCAS0771	2,22	putative adenylosuccinate synthetase	
BCAS0412	2,22	putative membrane protein	

BCAL3187	2,22	putative oxidoreductase		BCAL2607	0,38	putative exported protein	
BCAM1005	2,21	putative acyltransferase		BCAM2504	0,38	conserved hypothetical protein	
BCAL1551	2,20	ROK family regulatory protein		BCAL1622	0,37	molybdenum-pterin binding protein II	
BCAS0260	2,19	hypothetical protein		BCAM0628	0,37	putative low molecular weight phosphotyrosine	
BCAL3007	2,18	conserved hypothetical protein		BCAL1273	0,37	phosphate ABC transporter ATP-binding protein	
BCAM2227	2,18	putative pyochelin biosynthetic protein PchG		BCAL1113	0,37	ABC transporter ATP-binding protein	
BCAL1542	2,13	TetR family regulatory protein		BCAM1195	0,37	two-component regulatory system, sensor kinase	
BCAM0197	2,12	LysR family regulatory protein		BCAM2679	0,36	conserved hypothetical protein	
BCAM1672	2,11	mannonate dehydratase		BCAM2723	0,36	putative outer membrane porin protein	
BCAM2169	2,10	putative outer membrane autotransporter		BCAL0696	0,36	putative 2',3'-cyclic-nucleotide	
BCAL1722	2,07	putative exported chitinase	**1**	BCAM2498	0,35	extracellular solute-binding protein	
BCAS0496	2,04	benzoate 1,2-dioxygenase alpha subunit		BCAL1674	0,35	multidrug efflux system AmrA protein	
BCAM0476a	2,04	hypothetical protein		BCAL3473	0,34	putative outer membrane porin	
BCAL1806	2,04	conserved hypothetical protein		BCAL1274	0,34	phosphate transport system-related protein	
BCAL1524	2,04	putative lipoprotein		BCAM0502	0,34	conserved hypothetical protein	
BCAS0739	2,04	putative acetyl-CoA synthetase		BCAL1117	0,33	LacI family regulatory protein	
BCAM1927	2,01	putative exported protein		BCAM2010	0,33	conserved hypothetical protein	
BCAM0695	2,00	putative lipoprotein		BCAL2819	0,32	putative permease protein	
BCAM2725	0,50	putative oligopeptide ABC transporter		BCAL1271	0,31	phosphate transport system permease protein	
BCAL2598	0,49	putative DNA-binding protein		BCAM2009	0,25	2OG-Fe(II) oxygenase superfamily protein	
BCAM2623	0,49	conserved hypothetical protein		BCAL1083	0,25	putative exported alkaline phosphatase	
BCAM2489	0,48	putative transmembrane phosphate transporter		BCAM2007	0,23	TonB-dependent siderophore receptor	
BCAL2050	0,48	4Fe-4S ferredoxin		BCAM1187	0,13	TonB-dependent siderophore receptor	**5**
BCAM1376	0,48	putative porin					
BCAL0722	0,48	C4-dicarboxylate transport protein		IG2_2089610	73,40	interG_chr2_pos_1036_2089610:2090371	
BCAM1405	0,47	levansucrase		IG1_1836014	9,43	interG_chr1_pos_1002_1836014:1836102	
BCAL1619	0,47	alkanesulfonate monooxygenase		IG2_2087974	9,06	interG_chr2_pos_1034_2087974:2088183	
BCAM0391	0,47	putative exported protein		IG2_230868	7,05	interG_chr2_pos_110_230868:232940	
BCAM1777A	0,47	putative exported protein		IG2_2589126	6,46	interG_chr2_pos_1238_2589126:2589204	
BCAL1620	0,46	putative aliphatic sulfonates transport permease		IG3_811123	5,16	interG_chr3_pos_363_811123:815031	
BCAL1688	0,45	sigma factor 70 EcfI (OrbS)		IG1_3476475	4,67	interG_chr1_pos_1693_3476475:3476645	
BCAM1198	0,45	conserved hypothetical protein		IG1_2117018	4,20	interG_chr1_pos_1109_2117018:2120766	
BCAS0257	0,45	putative acetyltransferase		IG1_3297972	3,99	interG_chr1_pos_1609_3297972:3298473	
BCAL0124	0,45	flagellar regulon master regulator subunit FlhD		IG1_901672	3,96	interG_chr1_pos_504_901672:902168	
BCAL3001	0,45	TonB-dependent siderophore receptor		IG2_219668	3,59	interG_chr2_pos_104_219668:220648	
BCAM0776	0,45	putative cNMP-binding domain protein		IG1_2653432	2,98	interG_chr1_pos_1304_2653432:2653684	
BCAL1063	0,45	succinylarginine dihydrolase		IG2_1926503	2,94	interG_chr2_pos_955_1926503:1926918	
BCAM1745	0,44	putative magnesium-transporting ATPase		IG1_2605106	2,64	interG_chr1_pos_1279_2605106:2605488	
BCAM1283	0,44	putative phosphoesterase		IG2_1120955	2,62	interG_chr2_pos_580_1120955:1123103	
BCAM2060	0,44	putative divalent cation transporter		IG1_1814299	2,58	interG_chr1_pos_989_1814299:1814586	
BCAL2049	0,44	fumarate reductase/succinate dehydrogenase		IG2_943874	2,46	interG_chr2_pos_486_943874:943947	
BCAM1804	0,44	methyl-accepting chemotaxis protein		IG3_289386	2,38	interG_chr3_pos_152_289386:289561	
BCAL1114	0,43	putative ABC transport system, membrane protein		IG2_1853918	2,35	interG_chr2_pos_929_1853918:1854189	
BCAM1199	0,43	two-component regulatory system, response		IG1_680158	2,34	interG_chr1_pos_393_680158:681471	
BCAL1272	0,42	phosphate transport system permease protein		IG1_2651825	2,31	interG_chr1_pos_1303_2651825:2652279	
BCAM1282	0,41	putative di-haem cytochrome c peroxidase		IG2_840130	2,28	interG_chr2_pos_425_840130:840302	
BCAM1197	0,41	conserved hypothetical protein		IG1_2166257	2,24	interG_chr1_pos_1130_2166257:2171209	
BCAL1275	0,40	phosphate regulon two-component regulatory		IG1_1224302	2,24	interG_chr1_pos_668_1224302:1224367	
BCAL1270	0,39	phosphate transport system, substrate-binding		IG1_3180610	2,24	interG_chr1_pos_1539_3180610:3180736	
BCAM1149	0,39	putative lipoprotein	**5**	IG2_2036821	2,22	interG_chr2_pos_1014_2036821:2037459	

IG3_736569	2,21	interG_chr3_pos_333_736569:744787
IG1_48129	2,11	interG_chr1_pos_34_48129:48505
IG1_846330	2,08	interG_chr1_pos_482_846330:846415
IG2_836365	2,00	interG_chr2_pos_424_836365:839355
IG2_3061820	0,50	interG_chr2_pos_1426_3061820:3063511
IG2_2970010	0,40	interG_chr2_pos_1386_2970010:2971563
IG2_2227257	0,32	interG_chr2_pos_1118_2227257:2227324
IG1_3089813	0,32	interG_chr1_pos_1515_3089813:3093964
BCALr0472	5,21	tRNA Phe anticodon GAA, Cove score 86.97
BCALr0800	4,64	tRNA Gln anticodon TTG, Cove score 73.12
BCALr0218c	4,57	tRNA Thr anticodon GGT, Cove score 90.12
BCALr3029	3,73	tRNA Ser anticodon CGA, Cove score 71.72
BCALr0457	3,70	tRNA Lys anticodon CTT, Cove score 94.68
BCALr1551a	3,43	tRNA Leu anticodon CAG, Cove score 72.78
BCALr0409c	3,14	tRNA Ala anticodon TGC, Cove score 90.89
BCALr1625	2,97	tRNA His anticodon GTG, Cove score 78.40
BCALr2852a	2,78	tRNA Cys anticodon GCA, Cove score 64.58
BCALr1899	2,76	Bacterial signal recognition particle RNA (RF00169)
BCALr2281	2,75	tRNA Ser anticodon GGA, Cove score 70.94
BCALr0080	2,74	Perfect repeat flanking prophage
BCALr2344	2,69	tRNA Leu anticodon GAG, Cove score 63.68
BCALr2006	2,66	tRNA Leu anticodon TAG, Cove score 75.32
BCALr3009b	2,58	tRNA Arg anticodon ACG, Cove score 87.10
BCALr1290	2,46	Perfect repeat flanking genomic island
BCALr3075	2,41	Perfect repeat flanking genomic island
BCALr2125e	2,21	tRNA Asp anticodon GTC, Cove score 95.34
BCALr2458	2,13	tRNA Arg anticodon CCT, Cove score 72.32
BCALr3205	2,09	tRNA Lys anticodon TTT, Cove score 90.90
BCALr1614	2,08	tRNA Met anticodon CAT, Cove score 86.01
BCALr1279	2,06	tRNA Pro anticodon TGG, Cove score 89.00
BCALr2219	2,02	tRNA Met anticodon CAT, Cove score 86.80
BCAMr1592	0,48	Cobalamin riboswitch as predicted by Rfam, RF00174

Table A2. Genes and ORFs, tRNA and intergenetic regions which are more than 2-fold differentially regulated in the H111-R (*cepR*$^+$) transcriptome versus the H111 wild type. [a)]: Genes in bold type illustrate QS-regulated genes also identified in the transcriptome analysis of *B. cenocepacia* K56-2 (O´Grady *et al*., 2009) and [b)] : QS- regulated genes identified by alternative approaches. References are: **1**: (Huber *et al*., 2001), **2**: (Riedel *et al*., 2003), **3**: (Huber *et al*., 2004), **4**: (Weingart *et al.*, 2005) **5**: (Chambers *et al*., 2006), **6**: (Subsin *et al*., 2007), **7**: (Malott *et al*., 2009) and **8**: (Ryan *et al.*, 2009).

Gene name [a)]	Fold change	Description	Reference [b)]
BCAM2062	4,97	conserved hypothetical protein	
BCAL0566	4,87	basal-body rod modification protein FlgD	
BCAL0140	4,79	flagellar biosynthetic protein FlhB	
BCAL3507	4,78	flagellar FliL protein	
BCAL0523	4,37	flagellar assembly protein FliH	
BCAL3501	4,10	flagellar biosynthetic protein FliR	
BCAM1777A	4,09	putative exported protein	
BCAL0142	4,06	putative flagellar biosynthesis protein	
BCAL0564	4,02	flagellar basal body rod protein FlgB	
BCAL0042	4,01	trifunctional transcriptional regulator PutA	
BCAL0144	4,00	RNA polymerase sigma factor for flagellar	
BCAL3505	3,99	flagellar motor switch protein FliN	
BCAL1731	3,98	Major Facilitator Superfamily protein	
BCAM1804	3,76	methyl-accepting chemotaxis protein	
BCAL3187	3,69	putative oxidoreductase	
BCAL0110	3,67	putative aminotransferase	
BCAL0571	3,66	flagellar basal body P-ring protein	
BCAL3506	3,56	flagellar motor switch protein FliM	**6**
BCAL1056	3,55	histidine transport system permease protein	
BCAL0522	3,54	flagellum-specific ATP synthase FliI	
BCAL0568	3,48	flagellar basal body rod protein FlgF	
BCAL0143	3,41	putative flagellar biosynthesis protein	
BCAL0528	3,37	conserved hypothetical protein	
BCAL0572	3,36	flagellar rod assembly protein/muramidase FlgJ	
BCAL1818	3,35	metallo-beta-lactamase superfamily protein	
BCAL1055	3,32	histidine transport system permease protein	
BCAM2021	3,27	methyl-accepting chemotaxis protein	
BCAM0778	3,22	OmpA family protein	
BCAL1500	3,21	conserved hypothetical protein	
BCAL2705	3,14	ABC transporter ATP-binding protein	
BCAL0200	3,14	putative lipoprotein	
BCAL0570	3,12	flagellar basal body L-ring protein	
BCAM1097	3,12	putative membrane protein	
BCAL0526	3,11	flagellar hook-basal body complex protein FliE	
BCAL0569	3,11	flagellar basal body rod protein FlgG	
BCAM1745	3,03	putative magnesium-transporting ATPase	
BCAL0525	3,02	flagellar M-ring protein FliF	
BCAL2706	2,97	ABC transporter ATP-binding protein	
BCAM1811	2,97	conserved hypothetical protein	
BCAL0524	2,97	flagellar motor switch protein FliG	
BCAM0776	2,94	putative cNMP-binding domain protein	
BCAL0521	2,92	flagellar FliJ protein	**8**
BCAL2708	2,92	putative amino-acid transport permease protein	
BCAL0668	2,91	serine peptidase, family S9 unassigned	
BCAL0520	2,90	putative flagellar hook-length protein FliK	
BCAL0198	2,90	putative outer membrane protein	
BCAL0660	2,89	biotin carboxylase	
BCAM1378	2,88	ABC transporter permease protein	
BCAL0561	2,87	flagella synthesis protein FlgN	
BCAL1819	2,81	conserved hypothetical protein	
BCAL2615	2,80	putative exported outer membrane porin	
BCAM1377	2,78	ABC transporter ATP-binding protein	
BCAL2707	2,72	putative transport system permease protein	
BCAL0128	2,71	chemotaxis two-component response regulator	
BCAL0766	2,70	putative branched-chain amino acid transport	
BCAM2060	2,70	putative divalent cation transporter	
BCAL2819	2,69	putative permease protein	
BCAL2905	2,68	hypothetical protein	
BCAL0567	2,67	flagellar hook protein 1 FlgE1	
BCAM2374	2,67	putative methyl-accepting chemotaxis protein	
BCAL0043	2,66	putative extracellular ligand-binding protein	
BCAL2709	2,66	conserved hypothetical protein	
BCAM1488	2,66	putative proline racemase	
BCAL0126	2,65	chemotaxis protein MotA	
BCAL3237	2,64	putative transposase	
BCAM1166	2,63	putative membrane protein	
BCAM0613	2,63	ABC transporter ATP-binding protein	
BCAL1142	2,61	putative transposase	

BCAM1744	2,61	serine peptidase, family S9		BCAM0749	2,30	lysine-specific permease	
BCAL2028	2,61	LysR family regulatory protein		BCAL0584	2,29	putative outer membrane porin protein	
BCAL2246	2,59	histidine ammonia-lyase		BCAL2741	2,29	putative exported protein	
BCAM1768	2,56	conserved hypothetical protein		BCAL0065	2,28	conserved hypothetical protein	
BCAL0527	2,56	flagellar protein FliS		BCAM1167	2,26	conserved hypothetical protein	
BCAL1549	2,54	putative sugar ABC transport system,		BCAM1205	2,26	putative membrane protein	
BCAM2502	2,53	3-dehydroquinate dehydratase		BCAL3040	2,25	ABC transporter, membrane permease	
BCAM2679	2,52	conserved hypothetical protein		BCAM2746	2,25	carbon starvation protein A	
BCAM0163	2,50	putative microcin immunity protein		BCAM2723	2,22	putative outer membrane porin protein	
BCAL0665	2,49	dethiobiotin synthetase		BCAM1376	2,20	putative porin	
BCAM2201	2,48	conserved hypothetical protein		BCAL2833	2,20	putative membrane protein	
BCAM0130	2,47	putative acyl-CoA dehydrogenase	**6**	BCAS0151	2,20	hypothetical protein	
BCAM0603	2,47	conserved hypothetical protein		BCAL0576	2,19	flagellar hook-associated protein 1 (HAP1)	
BCAL0659	2,46	allophanate hydrolase subunit 1		BCAL0529	2,18	conserved hypothetical protein	
BCAL0287	2,46	putative outer membrane protein		BCAL3183	2,17	putative hydrolase	
BCAL0124	2,45	flagellar regulon master regulator subunit FlhD		BCAM1315	2,16	acylamide amidohydrolase	
BCAS0498	2,43	muconate cycloisomerase I 2		BCAM2063	2,16	putative carbohydrate-selective porin	
BCAL0132	2,43	chemotaxis protein methyltransferase		BCAM0761	2,16	histidine transport system permease	
BCAL3499	2,42	ABC transporter ATP-binding protein		BCAM0341	2,15	FMN-dependent NADH-azoreductase	**6**
BCAM2730	2,41	putative tripeptide permease		BCAL1635a	2,15	putative exported protein	
BCAL0691	2,41	putative cytidylyltransferase		BCAL0661	2,15	putative biotin-binding protein	
BCAL1547	2,40	carbohydrate kinase		BCAL0726	2,15	putative lipoprotein	
BCAM2685	2,40	conserved hypothetical protein		BCAM2482	2,14	agmatinase	
BCAL0762	2,39	putative methyl-accepting chemotaxis protein		BCAL2791	2,13	putative kynureninase	
BCAL0127	2,39	chemotaxis protein MotB		BCAL3473	2,13	putative outer membrane porin	
BCAL0133	2,38	put. chemoreceptor glutamine deamidase cheD		BCAM1810	2,12	putative cold shock protein	
BCAL0129	2,38	chemotaxis two-component sensor kinase CheA		BCAL2904	2,11	conserved hypothetical protein	
BCAS0050	2,37	putative amidohydrolase		BCAL1550	2,10	putative sugar ABC transporter ATP-binding	**6**
BCAM1165	2,37	putative membrane protein	**6**	BCAM2563	2,10	methyl-accepting chemotaxis protein	
BCAL3184	2,36	homogentisate 1,2-dioxygenase		BCAM1957	2,10	ABC transporter ATP-binding protein	
BCAM2501	2,35	shikimate 5-dehydrogenase		BCAM2500	2,09	putative glucarate transporter	
BCAM1831	2,35	putative cyclase		BCAL3186	2,09	conserved hypothetical protein	
BCAL0111	2,34	putative TPR repeat protein	**6**	BCAL3039	2,09	ABC transporter, membrane permease	
BCAM2720	2,34	putative phospholipase C		BCAL0767	2,08	ABC transporter ATP-binding protein	
BCAM2327	2,34	AraC family regulatory protein		BCAL2740	2,08	high-affinity nickel transport protein	
BCAM1286	2,33	secondary glycine betaine transporter betu		BCAS0236	2,07	putative haemagglutinin-related autotransporter	
BCAL0125	2,32	flagellar regulon master regulator subunit FlhC		BCAM1460	2,06	Major Facilitator Superfamily protein	
BCAL3001	2,32	TonB-dependent siderophore receptor		BCAM1769	2,06	putative D-alanyl-D-alanine dipeptidase	
BCAM2836	2,32	putative diguanylate cyclase		BCAS0398	2,06	putative diguanylate cyclase	
BCAL0134	2,31	chemotaxis-specific methylesterase		BCAL0413	2,06	conserved hypothetical protein	
BCAL0662	2,30	LamB/YcsF family protein		BCAM2171	2,05	putative rod shape-determining protein	

BCAL0950	2,05	Major Facilitator Superfamily protein		BCAM2042	0,47	type III secretion system protein	
BCAL3317	2,05	putative membrane protein		BCAM0541	0,47	putative DNA-binding protein	
BCAL3502	2,04	flagellar biosynthetic protein FliQ		BCAS0258	0,46	GntR family regulatory protein	
BCAL0768	2,04	conserved hypothetical protein		BCAM2286	0,46	putative short chain dehydrogenase	
BCAL2245	2,04	putative histidine utilization repressor		BCAL1573	0,46	hypothetical phage protein	
BCAL0113	2,03	B-type flagellar hook-associated protein 2		BCAM2289	0,46	conserved hypothetical protein	
BCAL0577	2,03	flagellar hook-associated protein 3 (HAP3)		BCAM0863	0,46	putative glycosyltransferase	
BCAL1270	2,02	phosphate transport system, substrate-binding		BCAM2233	0,46	putative pyochelin biosynthetic protein PchC	
BCAL1119	2,02	conserved hypothetical protein		BCAM1004	0,46	GCP-mannose 4,6-dehydratase	
BCAS0630	2,02	putative transporter - NRAMP family		BCAL0002	0,46	carboxylate-amine ligase YbdK	
BCAL2370	2,02	putative membrane protein		BCAS0480	0,46	putative exported protein	
BCAS0247	2,01	hypothetical protein		BCAM2049	0,45	type III secretion system protein	
BCAM2739A	0,50	hypothetical protein		BCAM2340	0,45	putative (R)-3-hydroxydecanoyl-ACP:CoA	
BCAM2169	0,50	putative outer membrane autotransporter		BCAM2051	0,45	type III secretion system protein	
BCAL1085	0,50	putative membrane protein		BCAL2512	0,45	putative membrane protein	
BCAS0773	0,50	putative exported protein		BCAM0843	0,45	putative lipoprotein	
BCAL1833	0,50	putative exported protein		BCAM0227	0,45	hybrid two component system kinase-response	
BCAM2041	0,49	type III secretion system protein BcscR	**6**	BCAL2206	0,44	phasin-like protein	
BCAM1800	0,49	conserved hypothetical protein		BCAL2301	0,44	putative exported protein	**6**
BCAM0549	0,49	10 kDa chaperonin 2		BCAM2779	0,44	putative N-isopropylammelide isopropyl	
BCAM1175	0,49	putative iron-sulfur cluster protein		BCAM0214	0,44	putative glycosyltransferase	
BCAM1411	0,49	putative short-chain dehydrogenase		BCAM1742	0,44	putative exported protein	
BCAM1414	0,49	conserved hypothetical protein		BCAM0853	0,44	conserved hypothetical protein	
BCAL2472	0,49	alpha,alpha-trehalose-phosphate synthase		**BCAL0831**	0,44	putative storage protein	
BCAM1012	0,48	putative histone-like protein		BCAM0652	0,44	putative gram-negative porin	
BCAM0633	0,48	conserved hypothetical protein		BCAL1664	0,44	conserved hypothetical protein	
BCAM0015	0,48	conserved hypothetical protein		BCAM0213	0,44	putative membrane protein	
BCAM2446	0,48	putative gram-negative porin		BCAM0857	0,44	phosphotyrosine phosphatase BceD	
BCAS0001	0,48	conserved hypothetical protein		**BCAM1871**	0,44	conserved hypothetical protein	
BCAM0952	0,48	ABC transporter ATP-binding protein		BCAL3322	0,44	putative DNA-binding protein	
BCAL2218	0,48	putative transposase		BCAM2224	0,43	putative pyochelin receptor protein FptA	
BCAL0193	0,48	putative exported protein		BCAL1297	0,43	hypothetical protein	
BCAS0479	0,48	conserved hypothetical protein		BCAM2378	0,43	putative Xaa-Pro dipeptidyl-peptidase	
BCAS0732	0,48	putative cytosine/purines, uracil, thiamine,		BCAM0906	0,43	putative dienelactone hydrolase family protein	
BCAM2385	0,48	rifampin ADP-ribosyl transferase		BCAM2034	0,43	putative UDP-glucose 6-dehydrogenase	
BCAM1066	0,48	hypothetical phage protein		**BCAL1689**	0,43	MbtH-like protein OrbH	
BCAM0507	0,47	CsbD-like protein		**BCAL3298**	0,43	conserved hypothetical protein	
BCAM0262	0,47	ABC transporter ATP-binding protein		BCAS0658	0,43	putative transposition-related protein	
BCAS0260	0,47	hypothetical protein		BCAL1294	0,42	conserved hypothetical protein	
BCAM2477	0,47	serine peptidase, family S10		BCAM2140	0,42	transporter system transport protein	
BCAM1358	0,47	gluconate dehydrogenase cytochrome c subunit		**BCAL0834**	0,42	putative membrane protein	

BCAL1513	0,42	MarR family regulatory protein	**6**	BCAL1974	0,31	putative lipoprotein	
BCAS0636	0,42	conserved hypothetical protein		BCAL1372	0,31	putative exported protein	
BCAL0926	0,42	putative glycerol-3-phosphate dehydrogenase		BCAM0031	0,30	conserved hypothetical protein	
BCAM0189	0,42	AraC family regulatory protein	**7**	**BCAM0184**	0,30	lectin	
BCAL3229	0,42	conserved hypothetical protein		BCAM0548	0,30	60 kDa chaperonin 2	
BCAL3006	0,42	cold shock-like protein	**6**	BCAM0634	0,30	hypothetical protein	
BCAL2786	0,41	putative selenium-binding protein		BCAM0854	0,30	bifunct. exopolysaccharide biosynthesis BceA	
BCAM0663	0,41	putative gram-negative porin		BCAL2290	0,29	putative bacterioferritin ferredoxin protein	
BCAM2336	0,41	putative sugar transferase		BCAM1964	0,29	putative exported protein	
BCAL0683	0,41	conserved hypothetical protein		BCAS0053	0,29	FMN reductase	
BCAM0810	0,40	putative aromatic oxygenase		**BCAM0191**	0,29	putative non-ribosomal peptide synthetase	**7**
BCAL0531	0,40	putative membrane protein		**BCAS0409**	0,29	zinc metalloprotease ZmpA	**5;6;7**
BCAM0021	0,40	putative patatin-like phospholipase		BCAM0803	0,29	muconolactone delta-isomerase	
BCAM2050	0,40	type III secretion system protein		BCAM0835	0,29	AraC family regulatory protein	
BCAL0358	0,40	metallo peptidase, family M1	**5**	**BCAL1688**	0,28	sigma factor 70 EcfI (OrbS)	
BCAM1454	0,39	putative exported protein		BCAM0859	0,28	tyrosine autokinase BceF	
BCAL2353	0,39	putative sulfate transporter		BCAM0804	0,28	catechol 1,2-dioxygenase 1	
BCAS0153	0,39	conserved hypothetical protein		BCAL1151	0,26	putative short-chain dehydrogenase	
BCAS0341	0,39	putative binding-protein-dependent transport		BCAM0844	0,26	conserved hypothetical protein	
BCAL3179	0,38	probable D-lactate dehydrogenase		BCAM0392	0,25	putative acetyltransferase	**5**
BCAM0096	0,38	ABC transporter ATP-binding protein		BCAM2771	0,25	putative dihydrodipicolinate synthetase	
BCAL0282	0,38	putative ABC transporter extracellular solute-		BCAS0638	0,25	10 kDa chaperonin 3	
BCAM2143	0,38	cable pilus associated adhesin protein		BCAL1813	0,24	multidrug efflux system outer membrane protein	
BCAM1959	0,38	putative membrane protein		BCAM0030	0,24	conserved hypothetical protein	
BCAL1952	0,37	conserved hypothetical protein		BCAM2770	0,23	putative sulfur-binding protein	
BCAS0323	0,37	LacI family regulatory protein		BCAM0188	0,22	N-acylhomoserine lactone dependent regulatory	**7**
BCAM1540	0,37	putative dehydrogenase, molybdopterin binding		**BCAL0833**	0,22	putative Acetoacetyl-CoA reductase	
BCAL1665	0,37	SpoVR like protein		BCAM1669	0,22	putative exported protein	
BCAM1861	0,36	calcineurin-like phosphoesterase		BCAM0504	0,20	CsbD-like protein	
BCAM2653	0,36	putative exported protein		BCAM0201	0,20	Major Facilitator Superfamily protein	**7**
BCAM0934	0,36	efflux system transport protein		BCAM0505	0,20	putative membrane-attached protein	
BCAS0637	0,35	60 kDa chaperonin 3		**BCAM0186**	0,20	lectin	**7**
BCAM0855	0,35	UDP-glucose dehydrogenase BceC		BCAM2052	0,19	putative type III secretion system protein	
BCAL2297	0,34	conserved hypothetical protein		BCAM2505	0,19	AraC family regulatory protein	
BCAL2352	0,34	putative carbonic anhydrase		BCAM0393	0,18	putative beta-lactamase, class D	
BCAM0861	0,34	putative glycosyltransferase		BCAM2308	0,18	putative leucyl aminopeptidase precursor	
BCAM1374	0,34	conserved hypothetical protein		BCAM0199	0,17	outer membrane efflux protein	**7**
BCAM0860	0,34	glycosyltransferase	**6**	BCAM0190	0,17	putative aminotransferase - class III	**7**
BCAM1010	0,32	putative UTP-glucose-1-phosphate		BCAM0192	0,16	conserved hypothetical protein	**7**
BCAL3321	0,32	glyoxalase/bleomycin resistance		BCAM0196	0,16	conserved hypothetical protein	**7**
BCAM1867	0,31	putative Mg(2+) transport ATPase (MgtC)		BCAM2053	0,16	putative type III secretion system protein	

BCAM2307	0,15	zinc metalloprotease ZmpB	**2;7**
BCAM0193	0,15	conserved hypothetical protein	**7**
BCAM0195	0,14	putative non-ribosomal peptide synthetase	**7**
BCAM0197	0,14	LysR family regulatory protein	
BCAS0292	0,13	conserved hypothetical protein	
BCAM0194	0,12	conserved hypothetical protein	**7**
BCAM0957	0,11	putative pepstatin-insensitive carboxyl	**5**
BCAM1413a	0,08	conserved hypothetical protein	
BCAM1412	0,07	conserved hypothetical protein	
BCAM0200	0,07	efflux system transport protein	**7**
BCAM1868	0,05	N-acylhomoserine lactone dependent regulatory	**5**
BCAS0293	0,04	nematocidal protein AidA	**2;3;4;5;6;7**
IG1_619308	5,51	interG_chr1_pos_364_619308:619404	
IG1_631117	5,16	interG_chr1_pos_374_631117:631394	
IG2_2295883	4,84	interG_chr2_pos_1135_2295883:2295972	
IG1_147730	4,25	interG_chr1_pos_68_147730:147909	
IG2_1187397	3,94	interG_chr2_pos_634_1187397:1190643	
IG2_840130	2,98	interG_chr2_pos_425_840130:840302	
IG1_3180610	2,90	interG_chr1_pos_1539_3180610:3180736	
IG1_679012	2,83	interG_chr1_pos_392_679012:679194	
IG1_3319801	2,81	interG_chr1_pos_1624_3319801:3321792	
IG3_811123	2,76	interG_chr3_pos_363_811123:815031	
IG2_1953120	2,71	interG_chr2_pos_965_1953120:1953814	
IG2_1299473	2,67	interG_chr2_pos_679_1299473:1300656	
IG1_52439	2,63	interG_chr1_pos_35_52439:52522	
IG1_3822505	2,63	interG_chr1_pos_1830_3822505:3822812	
IG2_836365	2,61	interG_chr2_pos_424_836365:839355	
IG2_831218	2,52	interG_chr2_pos_420_831218:831479	
IG2_187443	2,47	interG_chr2_pos_93_187443:192081	
IG2_671659	2,46	interG_chr2_pos_350_671659:674180	
IG1_3664357	2,44	interG_chr1_pos_1786_3664357:3666206	
IG1_582769	2,41	interG_chr1_pos_345_582769:583037	
IG1_2653432	2,37	interG_chr1_pos_1304_2653432:2653684	
IG3_536263	2,29	interG_chr3_pos_259_536263:537450	
IG3_319296	2,27	interG_chr3_pos_169_319296:321635	
IG1_3089813	2,27	interG_chr1_pos_1515_3089813:3093964	
IG1_3537906	2,26	interG_chr1_pos_1722_3537906:3539109	
IG1_3436208	2,22	interG_chr1_pos_1681_3436208:3448228	
IG2_1176685	2,22	interG_chr2_pos_619_1176685:1177107	
IG2_208795	2,20	interG_chr2_pos_100_208795:209162	
IG2_961669	2,19	interG_chr2_pos_497_961669:962112	
IG2_1985968	2,15	interG_chr2_pos_982_1985968:1986037	
IG1_579967	2,15	interG_chr1_pos_341_579967:580205	
IG1_830187	2,14	interG_chr1_pos_476_830187:830345	
IG1_3414831	2,13	interG_chr1_pos_1677_3414831:3418039	
IG2_2731412	2,13	interG_chr2_pos_1296_2731412:2731800	
IG1_2994853	2,12	interG_chr1_pos_1473_2994853:3000458	
IG1_155066	2,11	interG_chr1_pos_74_155066:155230	
IG1_603517	2,11	interG_chr1_pos_356_603517:606750	
IG1_2334236	2,09	interG_chr1_pos_1197_2334236:2334625	
IG1_825611	2,03	interG_chr1_pos_472_825611:825836	
IG1_2332334	2,01	interG_chr1_pos_1196_2332334:2332690	
IG2_219668	0,49	interG_chr2_pos_104_219668:220648	
IG2_439284	0,49	interG_chr2_pos_216_439284:439750	
IG2_2789309	0,48	interG_chr2_pos_1319_2789309:2790989	
IG3_27594	0,47	interG_chr3_pos_10_27594:27691	
IG2_2304176	0,47	interG_chr2_pos_1139_2304176:2304510	
IG2_2586922	0,44	interG_chr2_pos_1237_2586922:2587412	
IG1_1495942	0,43	interG_chr1_pos_805_1495942:1496283	
IG1_2166257	0,42	interG_chr1_pos_1130_2166257:2171209	
IG2_1565086	0,41	interG_chr2_pos_799_1565086:1565425	
IG2_2087974	0,40	interG_chr2_pos_1034_2087974:2088183	
IG2_949283	0,39	interG_chr2_pos_490_949283:949344	
IG2_2590456	0,38	interG_chr2_pos_1239_2590456:2590587	
IG2_943874	0,36	interG_chr2_pos_486_943874:943947	
IG2_2263493	0,35	interG_chr2_pos_1132_2263493:2263684	
IG1_2543049	0,31	interG_chr1_pos_1258_2543049:2543266	
IG2_230868	0,31	interG_chr2_pos_110_230868:232940	
IG1_901672	0,31	interG_chr1_pos_504_901672:902168	
IG2_2089610	0,31	interG_chr2_pos_1036_2089610:2090371	
IG2_1120955	0,30	interG_chr2_pos_580_1120955:1123103	
IG1_2935724	0,25	interG_chr1_pos_1444_2935724:2936297	
IG2_2589126	0,19	interG_chr2_pos_1238_2589126:2589204	
BCALr0472	6,88	tRNA Phe anticodon GAA, Cove score 86.97	
BCALr0457	6,43	tRNA Lys anticodon CTT, Cove score 94.68	
BCALr2006	5,41	tRNA Leu anticodon TAG, Cove score 75.32	
BCALr0080	5,26	Perfect repeat flanking prophage	
BCALr2852a	4,52	tRNA Cys anticodon GCA, Cove score 64.58	
BCALr0218c	4,46	tRNA Thr anticodon GGT, Cove score 90.12	
BCALr0800	4,43	tRNA Gln anticodon TTG, Cove score 73.12	

BCALr3029	4,37	tRNA Ser anticodon CGA, Cove score 71.72
BCALr0409c	4,21	tRNA Ala anticodon TGC, Cove score 90.89
BCALr1551a	4,07	tRNA Leu anticodon CAG, Cove score 72.78
BCALr2344	3,98	tRNA Leu anticodon GAG, Cove score 63.68
BCALr1614	3,89	tRNA Met anticodon CAT, Cove score 86.01
BCALr1625	3,75	tRNA His anticodon GTG, Cove score 78.40
BCALr2219	3,64	tRNA Met anticodon CAT, Cove score 86.80
BCALr3205	3,64	tRNA Lys anticodon TTT, Cove score 90.90
BCALr1492	3,54	tRNA Pro anticodon GGG, Cove score 81.38
BCALr2687	3,54	tRNA Leu anticodon CAA, Cove score 75.09
BCALr2994	3,51	tRNA Thr anticodon CGT, Cove score 87.03
BCALr2281	3,46	tRNA Ser anticodon GGA, Cove score 70.94
BCALr2145	3,29	tRNA Ser anticodon GCT, Cove score 69.97
BCALr3443	2,98	tRNA Pro anticodon CGG, Cove score 81.43
BCALr3075	2,90	Perfect repeat flanking genomic island
BCALr3009b	2,83	tRNA Arg anticodon ACG, Cove score 87.10
BCAMr0918	2,64	tRNA Met anticodon CAT, Cove score 71.40
BCALr1279	2,50	tRNA Pro anticodon TGG, Cove score 89.00
BCALr2125e	2,48	tRNA Asp anticodon GTC, Cove score 95.34
BCALr1899	2,45	Bacterial signal recognition particle RNA (RF00169)
BCALr1290	2,37	Perfect repeat flanking genomic island
BCALr0332	2,35	tRNA Thr anticodon TGT, Cove score 81.30
BCALr0949	2,21	tRNA Met anticodon CAT, Cove score 88.36
BCALr2852d	2,19	tRNA Gly anticodon GCC, Cove score 89.05
BCALr2125c	2,15	tRNA Asp anticodon GTC, Cove score 90.27
BCALr1993b	2,10	tRNA Val anticodon TAC, Cove score 94.99

Table A3. QS-regulated proteins which are more than 1.5-fold differentially expressed in mutant H111-I and/or mutant H111-R. (1257 proteins were identfied in total in analyses a and b).

Criteria: identified in the *cepI* and/or *cepR* mutant
extracellular or whole cell proteins
identified in analyses a and / or b
normalized factor = (√ factor a x factor b)

Abbreviations: EC (extracellular proteins)
WC (whole cell proteins = intracellular and surface-associated proteins)
CepR (H111-R mutant)
CepI (H111-I mutant)
WT (H111 wild type)

Differentially regulated in the proteome of H111-I and/or H111-R versus the H111 wild type

Differentially regulated in the proteome of H111-I and/or H111-R and the transcriptome of H111-R versus the H111 wild type

Extracellular proteins

Accession No.	Protein Name	Avg. iTRAQ Ratio 116/117* a	115/114* b	EC cepR / WT normalized	Avg. iTRAQ Ratio 115/117* a	116/114* b	EC cepI / WT normalized	Avg. iTRAQ Ratio 114/117* a	117/114* b	EC cepI+C8-HSL / WT normalized	Threshold reached ≥1.5 => up-regulated (1) ≤0.67=> down-regulated (-1) EC cepR	cepI
BCAL0009	pterin-4-alpha-carbinolamine dehydratase	4.763		4.763	2.032		2.032	1.721		1.721	1	1
BCAL0012	putative adenylate cyclase	3.219	2.796	3.000	1.089	1.498	1.277	0.853	1.119	0.977	1	0
BCAL0032 BCAL0036 (gi105894680)	ATP synthase B chain ATP synthase F1, beta subunit [*Burkholderia cenocepacia* AU 1054]	0.62		0.620	0.836		0.836	1.028		1.028	-1	0
BCAL0037	ATP synthase epsilon chain	1.525	1.808	1.660	0.631	0.843	0.729	0.656	0.913	0.774	1	0
BCAL0038 (gi117992142)	AMP-dependent synthetase and ligase [*Burkholderia phytofirmans* PsJN]	0.363	0.353	0.358	0.609	0.601	0.605	0.593	0.511	0.550	-1	-1
BCAL0043 BCAL0052 (gi117984267)	putative extracellular ligand-binding protein FAD linked oxidase-like [*Burkholderia phymatum* STM815]	3.573	2.972	3.259	0.935	1.105	1.016	1.403	1.335	1.369	1	0
BCAL0074	glycine cleavage system H protein	1.282	1.508	1.390	1.125	1.163	1.144	1.115	1.006	1.059	0	0
BCAL0077	putative oxidoreductase											
BCAL0080	putative cytochrome											
BCAL0095	putative phage major tail sheath protein	0.543		0.543	1.329		1.329	1.976		1.976	-1	0
BCAL0114 (gi105894597)	flagellin-like [*Burkholderia cenocepacia* AU 1054]	0.891	0.776	0.832	0.58	0.526	0.552	1.062	0.869	0.961	0	-1
BCAL0115	30S ribosomal protein S21 1	1.529	1.639	1.583	0.928	1.437	1.155	1.247	1.263	1.255	1	0

BCAL0122	histone-like nucleoid-structuring (H-NS)										
BCAL0154	histone-like nucleoid-structuring (H-NS)	5.66		5.660	1.66		1.660	1.358		1.358	1	1
BCAL0202	putative flavoprotein											
BCAL0210	TetR family regulatory protein											
BCAL0215	phenylacetic acid degradation protein PaaB	3.047	2.644	2.838	1.175	1.29	1.231	1.043	1.137	1.089	1	0
BCAL0216	phenylacetic acid degradation protein PaaA	1.626		1.626	1.571		1.571	1.534		1.534	1	1
BCAL0217 (gi52427635)	lipase/acylhydrolase, putative [*Burkholderia mallei* ATCC 23344]		0.455	0.455		1.461	1.461		0.699	0.699	-1	0
BCAL0221	transcription antitermination protein NusG	1.781		1.781	1.096		1.096	0.978		0.978	1	0
BCAL0224	50S ribosomal protein L10	0.507		0.507	1.167		1.167	1.139		1.139	-1	0
BCAL0231	elongation factor G	0.482	0.89	0.655	0.83	0.986	0.905	0.903	1.153	1.020	-1	0
BCAL0236	50S ribosomal protein L23	2.736		2.736	0.994		0.994	1.22		1.220	1	0
BCAL0237	50S ribosomal protein L2	0.525	0.741	0.624	0.838	0.904	0.870	1.117	1.237	1.175	-1	0
BCAL0238	30S ribosomal protein S19		1.951	1.951		0.831	0.831		1.367	1.367	1	0
BCAL0239	50S ribosomal protein L22											
BCAL0240	30S ribosomal protein S3	0.564	0.827	0.683	0.645	0.613	0.629	1.389	2.307	1.790	0	-1
BCAL0241	50S ribosomal protein L16	0.842		0.842	0.656		0.656	0.98		0.980	0	-1
BCAL0243	30S ribosomal protein S17	1.707	1.447	1.572	0.706	0.869	0.783	1.083	0.935	1.006	1	0
BCAL0245	50S ribosomal protein L24	2.289	2.725	2.498	0.706	0.89	0.793	0.954	1.228	1.082	1	0
BCAL0247	30S ribosomal protein S14	2.15	1.893	2.017	1.285	1.135	1.208	1.186	1.062	1.122	1	0
BCAL0249	50S ribosomal protein L6	3.825	3.688	3.756	1.145	1.381	1.257	1.165	1.253	1.208	1	0
BCAL0251	30S ribosomal protein S5	2.347	1.569	1.919	1.225	0.956	1.082	1.352	0.859	1.078	1	0
BCAL0253	50S ribosomal protein L15	1.551	1.467	1.508	0.876	0.837	0.856	1.269	1.167	1.217	1	0
BCAL0255	translation initiation factor IF-1	2.027	1.197	1.558	0.98	0.899	0.939	0.946	0.806	0.873	1	0
BCAL0258	30S ribosomal protein S11	1.528		1.528	1.234		1.234	1.102		1.102	1	0
BCAL0264	delta-aminolevulinic acid dehydratase	0.676		0.676	0.66		0.660	0.714		0.714	0	-1
BCAL0273	protein CyaY	1.783		1.783	1.184		1.184	0.99		0.990	1	0
BCAL0327	conserved hypothetical protein											
BCAL0332	putative stringent starvation protein B	2.566	1.776	2.135	0.709	1.098	0.882	0.634	0.732	0.681	1	0
BCAL0334	periplasmic solute-binding protein											
BCAL0341O	conserved hypothetical protein	2.534	4.499	3.376	1.573	1.682	1.627	1.325	1.985	1.622	1	1
BCAL0342	conserved hypothetical protein											
BCAL0343	conserved hypothetical protein 3	0.253	0.424	0.328	0.3	0.348	0.323	0.539	0.761	0.640	-1	-1
BCAL0347	protease associated ATPase ClpB											
BCAL0349	putative outer membrane protein											
BCAL0353	putative membrane protein	1.772	1.571	1.668	0.793	0.806	0.799	0.639	0.728	0.682	1	0
BCAL0358	metallo peptidase, family M1	0.273	0.52	0.377	0.275	0.521	0.379	0.836	0.976	0.903	-1	-1

BCAL0360	conserved hypothetical protein		1.687	1.687		1.142	1.142		0.968	0.968	1	0
BCAL0371	putative aromatic acid decarboxylase											
BCAL0387	putative GTP-binding protein											
BCAL0426	putative membrane protein	1.016	0.809	0.907	0.998	0.946	0.972	1.276	1.116	1.193	0	0
BCAL0482	putative rod shape-determining protein											
BCAL0487	endonuclease/exonuclease/phosphatase family	0.208		0.208	0.965		0.965	1.147		1.147	-1	0
BCAL0502	DksA/TraR C4-type zinc finger family protein	2.53	5.262	3.649	1.344	1.726	1.523	1.139	1.532	1.321	1	1
BCAL0503	putative cobalamin synthesis protein	0.616		0.616	1.177		1.177	0.969		0.969	-1	0
BCAL0507	diaminopimelate epimerase											
BCAL0529	conserved hypothetical protein											
BCAL0536	ferredoxin--NADP reductase	0.652		0.652	0.807		0.807	0.9		0.900	-1	0
BCAL0557	putative glutathione S-transferase protein											
BCAL0562	negative regulator of flagellin synthesis	0.827	0.886	0.856	0.605	0.639	0.622	0.558	0.469	0.512	0	-1
BCAL0565	flagellar basal-body rod protein FlgC	1.983	1.517	1.734	1.379	1.074	1.217	1.079	0.761	0.906	1	0
BCAL0567	flagellar hook protein 1 FlgE1	0.434	0.56	0.493	0.47	0.545	0.506	0.444	0.481	0.462	-1	-1
BCAL0568	flagellar basal-body rod protein FlgF	0.593	0.601	0.597	0.541	0.625	0.581	0.435	0.474	0.454	-1	-1
BCAL0569	flagellar basal-body rod protein FlgG	0.506	0.581	0.542	0.798	0.795	0.796	0.525	0.603	0.563	-1	0
BCAL0576	flagellar hook-associated protein 1 (HAP1)	0.436		0.436	0.588		0.588	0.342		0.342	-1	-1
BCAL0577	flagellar hook-associated protein 3 (HAP3)	0.62	0.71	0.663	0.794	0.871	0.832	0.645	0.711	0.677	-1	0
BCAL0616 (gi83653191)	uncharacterized ACR, COG1565 superfamily [*Burkholderia thailandensis* E264]											
BCAL0617	conserved hypothetical protein											
BCAL0626	putative 2-nitropropane dioxygenase											
BCAL0650	putative pyruvate-flavodoxin oxidoreductase		0.551	0.551		0.687	0.687		0.626	0.626	-1	0
BCAL0658	allophanate hydrolase subunit 2											
BCAL0677	thiol:disulfide interchange protein 7	2.045	1.807	1.922	1.284	1.248	1.266	1.419	1.324	1.371	1	0
BCAL0680	conserved hypothetical protein	4.913		4.913	2.118		2.118	1.37		1.370	1	1
BCAL0690	conserved hypothetical protein											
BCAL0703	serine peptidase, family S9											
BCAL0704	D-alanyl-D-alanine carboxypeptidase		1.063	1.063		1.123	1.123		1.481	1.481	0	0
BCAL0728	conserved hypothetical protein	1.836	1.564	1.695	2.148	1.489	1.788	0.773	1.039	0.896	1	1
BCAL0738	C-terminal processing protease-3	0.911	1.22	1.054	1.021	0.318	0.570	1.227	0.934	1.071	0	-1
BCAL0742	protein-export protein		0.489	0.489		1.289	1.289		1.278	1.278	-1	0
BCAL0763	putative exported protein	1.816	1.593	1.701	0.96	0.908	0.934	0.964	0.882	0.922	1	0
BCAL0764	putative exported protein											
BCAL0783	putative membrane protein											
BCAL0788	putative exported protein	0.433	0.973	0.649	1.248	1.975	1.570	1.178	2.503	1.717	-1	1

BCAL0794	conserved hypothetical protein											
BCAL0799	ribosomal L25p family protein	2.321	2.223	2.271	0.919	1.034	0.975	0.945	0.971	0.958	1	0
BCAL0800	ribose-phosphate pyrophosphokinase											
BCAL0804	putative membrane protein		4.307	4.307		2.035	2.035		1.444	1.444	1	1
BCAL0812	sigma-54 modulation protein	5.332	4.143	4.700	1.596	1.424	1.508	1.382	1.072	1.217	1	1
BCAL0817	putative 3-deoxy-D-manno-octulosonate											
BCAL0825	excinuclease ABC subunit A											
BCAL0831	putative storage protein											
BCAL0849	metallo peptidase, subfamily M48B											
BCAL0874	putative membrane protein	0.655	0.888	0.763	1.649	2.377	1.980	1.107	1.491	1.285	0	1
BCAL0878	conserved hypothetical protein	0.677	0.556	0.614	0.807	0.948	0.875	0.528	0.565	0.546	-1	0
BCAL0895	putative peptidyl-prolyl cis-trans isomerase	2.114	1.872	1.989	1.278	1.399	1.337	1.062	1.036	1.049	1	0
BCAL0926	putative glycerol-3-phosphate dehydrogenase											
BCAL0934	putative periplasmic cytochrome c containing	0.496		0.496	1.523		1.523	1.151		1.151	-1	1
BCAL0935	putative periplasmic cytochrome c protein	2.211		2.211	2.172		2.172	1.489		1.489	1	1
BCAL0965	putative hydrolase 1											
BCAL0980 (gi83653798)	molybdopterin biosynthesis moeA protein [*Burkholderia thailandensis* E264]	0.48		0.480	0.34		0.340	0.355		0.355	-1	-1
BCAL1044	GntR family regulatory protein	2.319		2.319	1.028		1.028	0.857		0.857	1	0
BCAL1065	periplasmic solute-binding protein	1.705	1.547	1.624	0.859	1.214	1.021	0.835	0.752	0.792	1	0
BCAL1072	conserved hypothetical protein		0.55	0.550		1.359	1.359		0.9	0.900	-1	0
BCAL1092	ABC transporter extracellular solute-binding	2.602		2.602	1.365		1.365	1.376		1.376	1	0
BCAL1215	dihydrolipoamide dehydrogenase											
BCAL1216 (gi116647358)	male sterility C-terminal domain [*Burkholderia cenocepacia* HI2424]	1.635		1.635	0.88		0.880	0.796		0.796	1	0
BCAL1259 (gi117988480)	hypothetical protein [*Burkholderia phytofirmans* PsJN]	0.422		0.422	1.148		1.148	0.706		0.706	-1	0
BCAL1262	carbamoyl-phosphate synthase large chain	0.52		0.520	1.084		1.084	1.261		1.261	-1	0
BCAL1263	transcription elongation factor	2.495		2.495	1.205		1.205	0.93		0.930	1	0
BCAL1394	putative exported protein											
BCAL1411	putative exported protein	0.593	0.672	0.631	0.627	0.699	0.662	0.42	0.476	0.447	-1	-1
BCAL1413	glutaminyl-tRNA synthetase	0.431		0.431	0.891		0.891	1.121		1.121	-1	0
BCAL1416	alanyl-tRNA synthetase											
BCAL1472	succinyl-CoA:3-ketoacid-coenzyme A transferase											
BCAL1478	putative hydrolase											
BCAL1482	translation initiation factor IF-3	1.634		1.634	0.911		0.911	0.912		0.912	1	0
BCAL1484	50S ribosomal protein L20	0.755		0.755	0.976		0.976	0.957		0.957	0	0
BCAL1486	phenylalanyl-tRNA synthetase beta chain		1.113	1.113		0.936	0.936		1.429	1.429	0	0

BCAL1496	putative exported protein	1.128		1.128	0.592		0.592	0.7		0.700	0	-1
BCAL1497 (gi91686933)	putative lipoprotein transmembrane [*Burkholderia xenovorans* LB400]		0.602	0.602		1.462	1.462		0.724	0.724	-1	0
BCAL1501 (gi117993520)	transcriptional regulator, LysR family [*Burkholderia phytofirmans* PsJN]											
BCAL1504	RNA pseudouridylate synthase family protein	0.531	0.37	0.443	1.142	0.628	0.847	0.916	0.912	0.914	-1	0
BCAL1506	N utilization substance protein A	1.967	2.386	2.166	1.284	1.319	1.301	1.134	1.445	1.280	1	0
BCAL1507	translation initiation factor IF-2	0.165	0.373	0.248	0.849	0.983	0.914	0.435	1.021	0.666	-1	0
BCAL1515	2-oxoglutarate dehydrogenase E1 component	0.444	0.61	0.520	0.867	0.913	0.890	0.941	1.172	1.050	-1	0
BCAL1530	flp pilus type assembly protein											
BCAL1611	dihydroorotate dehydrogenase											
BCAL1636	putative exported endonuclease											
BCAL1657	putative ribose transport system	1.919	1.728	1.821	0.983	0.951	0.967	1.09	0.823	0.947	1	0
BCAL1663	PrkA family serine protein kinase											
BCAL1675 (gi76580894)	TetR family regulatory protein [*Burkholderia pseudomallei* 1710b]	0.621	0.629	0.625	1.076	0.947	1.009	0.601	0.676	0.637	-1	0
BCAL1677	putative type-1 fimbrial protein	0.176	0.147	0.161	0.225	0.149	0.183	0.242	0.188	0.213	-1	-1
BCAL1696 (gi83650419)	nonribosomal peptide synthetase, putative [*Burkholderia thailandensis* E264]											
BCAL1722 (gi116647781)	chitinase [*Burkholderia cenocepacia* HI2424]	0.351	0.57	0.447	0.64	0.83	0.729	0.942	1.026	0.983	-1	0
BCAL1824	putrescine-binding periplasmic protein	1.561		1.561	0.89		0.890	1.314		1.314	1	0
BCAL1831	putative betaine aldehyde dehydrogenase	0.463		0.463	0.828		0.828	1.01		1.010	-1	0
BCAL1849	putative exported protein	0.57	0.664	0.615	0.69	0.816	0.750	0.556	0.693	0.621	-1	0
BCAL1850	putative dehydrogenase											
BCAL1868	conserved hypothetical protein	6.442	3.79	4.941	1.337	1.261	1.298	1.01	0.778	0.886	1	0
BCAL1869	putative exported protein	0.971	0.833	0.899	1.067	1.076	1.071	1.075	0.882	0.974	0	0
BCAL1872	putative nucleotide phosphoribosyltransferase											
BCAL1874	putative ATP phosphoribosyltransferase											
BCAL1879	Hfq protein	1.082	0.625	0.822	2.301	1.358	1.768	0.979	0.915	0.946	0	1
BCAL1892	RNA polymerase sigma factor	1.916		1.916	1.221		1.221	0.899		0.899	1	0
BCAL1898	conserved hypothetical protein											
BCAL1900	thioredoxin	1.578		1.578	1.59		1.590	1.396		1.396	1	1
BCAL1905	50S ribosomal protein L31	2.842	2.055	2.417	1.044	0.889	0.963	0.952	0.875	0.913	1	0
BCAL1917	putative exported protein	0.295	0.407	0.347	0.595	0.653	0.623	0.505	0.556	0.530	-1	-1
BCAL1919 (gi118654245)	ATPase AAA-2 [*Burkholderia cenocepacia* MC0-3]											
BCAL1934	UDP-glucuronic acid decarboxylase											
BCAL1942	50S ribosomal protein L9	3.276	2.508	2.866	1.45	1.338	1.393	1.87	1.453	1.648	1	0

BCAL1943	30S ribosomal protein S18	2.927	2.727	2.825	1.164	1.242	1.202	1.55	1.284	1.411	1	0
BCAL1945	putative 30S ribosomal protein S6	1.77	1.991	1.877	0.932	1.009	0.970	0.98	1.007	0.993	1	0
BCAL1946 (gi52427005)	conserved hypothetical protein [*Burkholderia mallei* ATCC 23344]											
BCAL1956	putative lipoprotein	0.561		0.561	1.425		1.425	1.017		1.017	-1	0
BCAL1961 (gi105898095)	ankyrin repeat protein [*Burkholderia cenocepacia* AU 1054]											
BCAL1964	putative thymidylate kinase											
BCAL1971	conserved hypothetical protein											
BCAL1982	peptide methionine sulfoxide reductase											
BCAL1985	putative exported isomerase	4.22	2.964	3.537	1.589	1.348	1.464	1.439	1.143	1.282	1	0
BCAL1992 (gi77967434)	lipolytic enzyme, G-D-S-L [*Burkholderia* sp. 383]		2.952	2.952		1.222	1.222		0.841	0.841	1	0
BCAL1995	ATP-dependent Clp protease ATP-binding subunit											
BCAL1996	ATP-dependent Clp protease proteolytic subunit	1.019		1.019	1.742		1.742	1.159		1.159	0	1
BCAL1997	trigger factor	3.307	3.539	3.421	0.999	1.235	1.111	1.227	1.236	1.231	1	0
BCAL1998	putative kinase											
BCAL2013	AhpC/TSA family protein	1.211	1.225	1.218	0.894	0.894	0.894	0.825	0.917	0.870	0	0
BCAL2018 (gi105898280)	transcriptional regulator, GntR family [*Burkholderia cenocepacia* AU 1054]	3.562		3.562	0.868		0.868	1.257		1.257	1	0
BCAL2022	PspA/IM30 family protein	3.133	2.65	2.881	1.26	0.776	0.989	0.82	0.933	0.875	1	0
BCAL2039	putative uricase											
BCAL2063	inosine-5'-monophosphate dehydrogenase	0.557	0.716	0.632	1.146	0.84	0.981	1.068	1.119	1.093	-1	0
BCAL2082	chaperone protein Skp precursor	1.92		1.920	1.297		1.297	0.671		0.671	1	0
BCAL2083 (gi118657256)	surface antigen (D15) [*Burkholderia multivorans* ATCC 17616]	0.53		0.530	1.243		1.243	0.882		0.882	-1	0
BCAL2088	ribosome recycling factor	2.439	2.03	2.225	1.075	1.072	1.073	1.371	0.931	1.130	1	0
BCAL2091	30S ribosomal protein S2	2.273	1.267	1.697	1.467	1.051	1.242	1.383	1.059	1.210	1	0
BCAL2094 (gi91688077)	pseudouridine synthase, Rsu [*Burkholderia xenovorans* LB400]	0.578	0.742	0.655	0.404	0.438	0.421	0.412	0.497	0.453	-1	-1
BCAL2098	putative chromosome partition protein		1.403	1.403		2.137	2.137		1.104	1.104	0	1
BCAL2110 (gi52427334)	alanine racemase [*Burkholderia mallei* ATCC 23344]											
BCAL2123	conserved hypothetical protein											
BCAL2126	glutamyl-tRNA synthetase											
BCAL2150	cysteinyl-tRNA synthetase											
BCAL2152	peptidyl-prolyl cis-trans isomerase A precursor	0.672	0.889	0.773	0.883	0.787	0.834	0.683	0.536	0.605	0	0
BCAL2166	putative lipoprotein	1.251		1.251	1.672		1.672	1.367		1.367	0	1
BCAL2180	putative 2-dehydro-3-deoxyphosphooctonate											
BCAL2189	peptide chain release factor 2	0.801	0.512	0.640	1.541	0.872	1.159	0.853	0.594	0.712	-1	0

BCAL2190 (gi117980152)	lysyl-tRNA synthetase [*Burkholderia phymatum* STM815]											
BCAL2206	phasin-like protein		0.498	0.498		0.862	0.862		1.66	1.660	-1	0
BCAL2209	pyruvate dehydrogenase E1 component	0.75	0.864	0.805	1.067	0.94	1.001	1.223	1.15	1.186	0	0
BCAL2211	two-component regulatory system, response	1.776	1.431	1.594	1.179	1.097	1.137	1.16	0.992	1.073	1	0
BCAL2226	putative molybdopterin-binding protein											
BCAL2288	bacterioferritin	0.864	1.237	1.034	1.42	1.664	1.537	1.029	1.253	1.135	0	1
BCAL2300	putative exported protein	0.876		0.876	0.459		0.459	0.554		0.554	0	-1
BCAL2329	NUDIX hydrolase											
BCAL2336	putative NADH dehydrogenase I chain I	1.163		1.163	1.856		1.856	1.545		1.545	0	1
BCAL2340	putative NADH dehydrogenase I chain E											
BCAL2342	NADH dehydrogenase I chain C	1.543	1.048	1.272	1.897	1.349	1.600	1.473	1.031	1.232	0	1
BCAL2349	30S ribosomal protein S15	1.767	1.335	1.536	0.939	0.941	0.940	1.322	0.977	1.136	1	0
BCAL2354	2-isopropylmalate synthase											
BCAL2359	acetolactate synthase isozyme III large subunit											
BCAL2384	quinone oxidoreductase	0.475	0.745	0.595	1.455	1.504	1.479	1.289	1.59	1.432	-1	0
BCAL2388	conserved hypothetical protein	2.166	1.531	1.821	1.558	0.907	1.189	1.766	1.154	1.428	1	0
BCAL2394 (gi52428872)	maf protein [*Burkholderia mallei* ATCC 23344]											
BCAL2396	conserved hypothetical protein	4.409	2.567	3.364	1.506	1.305	1.402	1.381	1.073	1.217	1	0
BCAL2401	putative exported protein	0.548	0.542	0.545	0.792	0.729	0.760	0.516	0.417	0.464	-1	0
BCAL2409 (gi121229043)	DNA polymerase III, alpha subunit, form 1 [*Burkholderia mallei* SAVP1]	0.994	0.839	0.913	0.736	0.591	0.660	0.637	0.579	0.607	0	-1
BCAL2415	phosphoribosylglycinamide formyltransferase 2											
BCAL2418	putative exported protein	0.605	0.593	0.599	1.206	0.773	0.966	0.918	0.791	0.852	-1	0
BCAL2429	putative cytochrome C precursor-related protein	1.995		1.995	1.462		1.462	1.019		1.019	1	0
BCAL2446	putative aminotransferase											
BCAL2476a	conserved hypothetical protein (fragment)	2.077	2.056	2.066	1.189	1.327	1.256	0.994	1.064	1.028	1	0
BCAL2606	two-component regulatory system, response		2.472	2.472		1.9	1.900		1.255	1.255	1	1
BCAL2607	putative exported protein		3.55	3.550		0.609	0.609		0.533	0.533	1	-1
BCAL2616	nitrogen regulatory protein P-II 2	1.53		1.530	1.523		1.523	1.133		1.133	1	1
BCAL2622	inorganic pyrophosphatase	1.929		1.929	1.366		1.366	1.223		1.223	1	0
BCAL2630	putative porphobilinogen deaminase protein											
BCAL2649	conserved hypothetical protein	1.992	1.514	1.737	1.478	1.1	1.275	0.913	0.902	0.907	1	0
BCAL2653	ParA family ATPase											
BCAL2657	putative bifunctional cobalamin biosynthesis											
BCAL2666	conserved hypothetical protein		1.474	1.474		1.311	1.311		0.681	0.681	0	0
BCAL2669	putative exported protein	1.195	1.28	1.237	0.948	0.929	0.938	1.023	0.818	0.915	0	0

BCAL2676	aminopeptidase A											
BCAL2700	putative oxidoreductase	1.201	1.167	1.184	2.071	1.236	1.600	1.676	1.294	1.473	0	1
BCAL2710	4-hydroxy-3-methylbut-2-enyl diphosphate		0.647	0.647		0.848	0.848		0.64	0.640	-1	0
BCAL2714	50S ribosomal protein L28	1.757	2.047	1.896	1.331	1.247	1.288	0.999	1.169	1.081	1	0
BCAL2732	cold shock-like protein	0.726	0.849	0.785	0.883	1.006	0.942	0.73	0.835	0.781	0	0
BCAL2733 (gi115282608)	multicopper oxidase, type 3 [*Burkholderia cepacia* AMMD]											
BCAL2736	isocitrate dehydrogenase	0.853		0.853	1.57		1.570	1.669		1.669	0	1
BCAL2754 (gi52429288)	chromate transport protein [*Burkholderia mallei* ATCC 23344]	1.572		1.572	0.727		0.727	0.691		0.691	1	0
BCAL2757	superoxide dismutase SodB	1.072		1.072	1.515		1.515	1.867		1.867	0	1
BCAL2762	putative adenylate kinase	1.877	2.291	2.074	0.914	1.167	1.033	0.981	0.958	0.969	1	0
BCAL2765	putative 30S ribosomal protein S20	2.278		2.278	1.387		1.387	1.986		1.986	1	0
BCAL2769	conserved hypothetical protein	1.713		1.713	1.174		1.174	0.951		0.951	1	0
BCAL2773 (gi117980128)	phage integrase [*Burkholderia phymatum* STM815]	1.627		1.627	1.705		1.705	1.329		1.329	1	1
BCAL2785	putative peptide methionine sulfoxide reductase	0.262	0.867	0.477	0.511	0.941	0.693	0.311	0.816	0.504	-1	0
BCAL2791	putative kynureninase	0.663	0.973	0.803	1.797	1.261	1.505	1.782	1.319	1.533	0	1
BCAL2796 (gi118659676)	aldehyde dehydrogenase [*Burkholderia multivorans* ATCC 17616]											
BCAL2821	RND family efflux system transporter protein	0.596		0.596	1.265		1.265	1.455		1.455	-1	0
BCAL2828	putative exported protein	0.427	0.453	0.440	0.548	0.544	0.546	0.451	0.426	0.438	-1	-1
BCAL2839	fructose-bisphosphate aldolase	0.949		0.949	1.134		1.134	1.347		1.347	0	0
BCAL2858	hypothetical protein	1.75		1.750	0.802		0.802	1.039		1.039	1	0
BCAL2864	hypothetical protein											
BCAL2888	ribonuclease E 1	0.532	0.396	0.459	1.268	1.015	1.134	1.114	0.655	0.854	-1	0
BCAL2911 (gi115281004)	conserved hypothetical protein [*Burkholderia cepacia* AMMD]											
BCAL2915	dihydrofolate reductase											
BCAL2929 (gi91690051)	L-sorbosone dehydrogenase [*Burkholderia xenovorans* LB400]											
BCAL2935	electron transfer flavoprotein beta-subunit	2.466	4.022	3.149	1.08	1.266	1.169	1.502	1.532	1.517	1	0
BCAL2941	putative exported transglycosylase											
BCAL2943	putative exported protein	0.363	0.353	0.358	0.609	0.601	0.605	0.593	0.511	0.550	-1	-1
BCAL2950	30S ribosomal protein S1	2.531	2.033	2.268	1.18	0.969	1.069	1.182	1.069	1.124	1	0
BCAL2954 (gi77966373)	chorismate mutase/Prephenate dehydratase [*Burkholderia* sp. 383]											
BCAL2958	putative ompA family protein	2.52	2.672	2.595	1.088	1.259	1.170	1.032	1.163	1.096	1	0
BCAL2961 (gi83650624)	integrase [*Burkholderia thailandensis* E264]	0	0.638	0.638	2.443	0.841	1.433	3.167	0.329	1.021	-1	0
BCAL2966	conserved hypothetical protein											

BCAL3006	cold shock-like protein	0.914	0.884	0.899	0.623	0.629	0.626	0.859	0.721	0.787	0	-1
BCAL3008	putative outer membrane porin protein	0.433	0.54	0.484	0.821	0.98	0.897	0.988	1.205	1.091	-1	0
BCAL3024 (gi105892254)	conserved hypothetical protein [*Burkholderia cenocepacia* AU 1054]	1.747		1.747	0.637		0.637	1.009		1.009	1	-1
BCAL3036	hypothetical protein											
BCAL3041	maltose-binding protein	1.11		1.110	0.99		0.990	1.041		1.041	0	0
BCAL3051	riboflavin synthase alpha chain											
BCAL3054	6,7-dimethyl-8-ribityllumazine synthase	1.627	2.912	2.177	1.03	1.216	1.119	1.07	0.546	0.764	1	0
BCAL3055	probable N utilization substance protein B		5.784	5.784		1.593	1.593		1.072	1.072	1	1
BCAL3111	conserved hypothetical protein	0.706	0.999	0.840	0.992	1.098	1.044	1.302	1.162	1.230	0	0
BCAL3113 (gi118651602)	Phosphomannomutase [*Burkholderia cenocepacia* MC0-3]	0.58		0.580	0.821		0.821	1.007		1.007	-1	0
BCAL3115 (gi117991085)	glycosyl transferase, family 2 [*Burkholderia phytofirmans* PsJN]											
BCAL3136 (gi118656806)	apaH, bis(5'nucleosyl)-tetraphosphatase, ApaH [*Burkholderia multivorans* ATCC 17616]											
BCAL3142	conserved hypothetical protein											
BCAL3146	60 kDa chaperonin 1	1.832	1.805	1.818	1.404	1.316	1.359	1.397	1.448	1.422	1	0
BCAL3147	10 kDa chaperonin 1	1.856	2.42	2.119	1.124	1.282	1.200	0.928	1.206	1.058	1	0
BCAL3166	putative lipoprotein	1.112		1.112	0.605		0.605	0.673		0.673	0	-1
BCAL3178	LysR family regulatory protein		0.642	0.642		0.611	0.611		0.888	0.888	-1	-1
BCAL3183	putative hydrolase											
BCAL3186	conserved hypothetical protein											
BCAL3191	putative glutaryl-CoA dehydrogenase											
BCAL3197	serine hydroxymethyltransferase	0.534	0.749	0.632	0.983	0.949	0.966	0.933	1.045	0.987	-1	0
BCAL3204	putative OmpA family lipoprotein	1.616	1.919	1.761	1.229	1.581	1.394	0.968	1.2	1.078	1	0
BCAL3205	putative exported protein	1.542	2.19	1.838	0.81	1.443	1.081	0.933	1.341	1.119	1	0
BCAL3220 (gi115285990)	beta-ketoacyl synthase [*Burkholderia cepacia* AMMD]											
BCAL3244 (gi83654978)	possible glycosyltransferase WbpX, putative [*Burkholderia thailandensis* E264]	2.315		2.315	0.984		0.984	1.197		1.197	1	0
BCAL3270	putative DnaK chaperone protein	2.355	2.135	2.242	1.022	1.084	1.053	1.233	1.132	1.181	1	0
BCAL3272	putative heat shock protein	2.509		2.509	1.634		1.634	1.006		1.006	1	1
BCAL3299	peroxidase/catalase KatB	0.44		0.440	0.89		0.890	1.096		1.096	-1	0
BCAL3316 (gi117985821)	protein of unknown function DUF330 [*Burkholderia phymatum* STM815]											
BCAL3332	aminopeptidase P											
BCAL3347	30S ribosomal protein S9	2.237	2.363	2.299	0.99	1.1	1.044	1.095	1.371	1.225	1	0
BCAL3358	periplasmic glutamate/aspartate-binding protein	2.809	3.404	3.092	1.255	1.327	1.290	1.207	1.497	1.344	1	0
BCAL3359	putative glutamate dehydrogenase	0.652		0.652	1.242		1.242	1.425		1.425	-1	0

BCAL3368 (gi117980776)	transcriptional regulator, RpiR family [*Burkholderia phymatum* STM815]		2.952	2.952		1.222	1.222		0.841	0.841	1	0
BCAL3370	gamma-glutamyl phosphate reductase											
BCAL3377	putative outer membrane protein											
BCAL3378	ferric uptake regulator	3.536	1.448	2.263	1.955	1.042	1.427	1.741	0.701	1.105	1	0
BCAL3388	glyceraldehyde 3-phosphate dehydrogenase 1	0.646		0.646	1.294		1.294	1.166		1.166	-1	0
BCAL3389	transketolase 1	0.482	0.857	0.643	0.967	1.094	1.029	1.151	1.133	1.142	-1	0
BCAL3402 (gi118641093)	short-chain dehydrogenase/reductase SDR [*Burkholderia ambifaria* MC40-6]	0.377		0.377	1.195		1.195	0.78		0.780	-1	0
BCAL3425	putative sugar kinase 3											
BCAL3426	putative lipoprotein	0.423		0.423	0.991		0.991	0.992		0.992	-1	0
BCAL3427	histone H1-like protein	2.854	4.975	3.768	0.858	1.68	1.201	1.159	1.899	1.484	1	0
BCAL3429	putative ribonucleoside reductase	0.439	0.589	0.508	0.884	1.01	0.945	1	1.101	1.049	-1	0
BCAL3436	prolyl-tRNA synthetase											
BCAL3467	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate											
BCAL3489 (gi76583601)	conserved hypothetical protein [*Burkholderia pseudomallei* 1710b]											
BCAL3507	flagellar FliL protein	1.979	1.301	1.605	2.01	1.135	1.510	1.467	1.235	1.346	1	1
BCAL3523	general secretory pathway protein G	1.115		1.115	1.596		1.596	0.945		0.945	0	1
BCAL3529	putative cobalamin synthesis protein/P47K											
BCAL3530	DNA-binding protein HU-alpha	6.238	6.765	6.496	1.049	1.296	1.166	1.355	1.329	1.342	1	0
BCAM0006	conserved hypothetical protein											
BCAM0026	putative siderophore-interacting protein											
BCAM0058	3-oxoadipate CoA-transferase subunit A											
BCAM0060 (gi52421938)	3-carboxy-cis,cis-muconate cycloisomerase [*Burkholderia mallei* ATCC 23344]											
BCAM0149 (gi77971729)	phospholipase D/Transphosphatidylase [*Burkholderia* sp. 383]	0.3	0.412	0.352	0.596	0.723	0.656	0.69	0.638	0.663	-1	-1
BCAM0173	ATP-independent RNA helicase											
BCAM0175 (gi77971682)	malate dehydrogenase (acceptor) [*Burkholderia* sp. 383]	0.086		0.086	0.168		0.168	0.18		0.180	-1	-1
BCAM0184	lectin											
BCAM0186	lectin	0.45	0.528	0.487	0.528	0.647	0.584	0.539	0.627	0.581	-1	-1
BCAM0276 (gi115285786)	UspA domain protein [*Burkholderia cepacia* AMMD]	1.486	1.66	1.571	1.162	1.237	1.199	1.132	1.439	1.276	1	0
BCAM0297 (gi115283858)	acetoacetyl-CoA reductase [*Burkholderia cepacia* AMMD]	0.642		0.642	1.174		1.174	0.774		0.774	-1	0
BCAM0313	putative exported protein	1.692		1.692	1.263		1.263	0.833		0.833	1	0
BCAM0357	LysR family regulatory protein	0.377	0.389	0.383	1.22	1.484	1.346	0.547	0.776	0.652	-1	0
BCAM0502	conserved hypothetical protein	1.86	1.498	1.669	1.429	1.167	1.291	1.447	1.107	1.266	1	0

BCAM0504	CsbD-like protein	3.716	3.463	3.587	1.042	1.095	1.068	0.933	1.043	0.986	1	0
BCAM0505	putative membrane-attached protein	1.312	2.559	1.832	0.503	1.002	0.710	0.689	1.027	0.841	1	0
BCAM0507	CsbD-like protein	2.993	2.908	2.950	0.596	0.763	0.674	1.814	1.373	1.578	1	0
BCAM0589	conserved hypothetical protein											
BCAM0600	conserved hypothetical protein		0.274	0.274		1.076	1.076		0.974	0.974	-1	0
BCAM0602 (gi134135913)	molecular chaperone-like protein [*Burkholderia vietnamiensis* G4]											
BCAM0630	putative dehydrogenase	0.951		0.951	1.671		1.671	0.884		0.884	0	1
BCAM0684	putative oxidoreductase											
BCAM0759 (gi134132844)	extracellular solute-binding protein, family 3 [*Burkholderia vietnamiensis* G4]											
BCAM0767 (gi117992836)	ABC transporter related [*Burkholderia phytofirmans* PsJN]											
BCAM0795 (gi117992171)	transcriptional regulator, MarR family [*Burkholderia phytofirmans* PsJN]	1.55		1.550	1.346		1.346	1.819		1.819	1	0
BCAM0811	putative aromatic oxygenase											
BCAM0829	putative riboflavin synthase alpha chain											
BCAM0832 (gi105893144)	Dyp-type peroxidase [*Burkholderia cenocepacia* AU 1054]											
BCAM0833 (gi118660931)	OsmC family protein [*Burkholderia multivorans* ATCC 17616]	2.833	2.619	2.724	1.599	1.465	1.531	1.126	1.063	1.094	1	1
BCAM0844	conserved hypothetical protein	2.969	3.316	3.138	1.362	1.408	1.385	1.3	1.142	1.218	1	0
BCAM0910	putative geranyltranstransferase											
BCAM0915	30S ribosomal protein S21 2	3.003	2.543	2.763	1.677	1.534	1.604	2.327	1.709	1.994	1	1
BCAM0916	conserved hypothetical protein	1.589		1.589	1.264		1.264	1.168		1.168	1	0
BCAM0917	putative DNA primase											
BCAM0953	extracellular solute-binding protein											
BCAM0961	aconitate hydratase	0.433	1.002	0.659	1.341	0.821	1.049	1.511	1.593	1.551	-1	0
BCAM0963	putative exported protein	2.112	2.268	2.189	0.936	1.165	1.044	1.04	1.006	1.023	1	0
BCAM0972	citrate synthase 1	0.605	0.885	0.732	0.482	0.728	0.592	0.581	0.773	0.670	0	-1
BCAM0986	aspartate-semialdehyde dehydrogenase											
BCAM0987	flagellar hook protein 2 FlgE2	0.436		0.436	0.471		0.471	0.454		0.454	-1	-1
BCAM0988	putative exported protein	0.771	0.959	0.860	0.9	1.034	0.965	0.861	0.579	0.706	0	0
BCAM1003	putative epimerase		0.908	0.908		0.644	0.644		0.799	0.799	0	-1
BCAM1012	putative histone-like protein	6.108	7.259	6.659	1.435	1.796	1.605	1.707	1.729	1.718	1	1
BCAM1015	putative porin	0.433	0.656	0.533	0.947	1.095	1.018	0.984	1.375	1.163	-1	0
BCAM1021	protein FdhE homologue											
BCAM1062	hypothetical phage protein	2.679		2.679	1.465		1.465	1.28		1.280	1	0
BCAM1111	ornithine decarboxylase	1	1.159	1.077	1.966	1.265	1.577	1.97	1.372	1.644	0	1

BCAM1123 (gi91692859)	hypothetical protein [*Burkholderia xenovorans* LB400]		0.615	0.615		1.227	1.227		0.835	0.835	-1	0
BCAM1189 (gi115283494)	transcriptional regulator, LysR family [*Burkholderia cepacia* AMMD]											
BCAM1204	alanine racemase, catabolic											
BCAM1234	conserved hypothetical protein	1.627		1.627	1.705		1.705	1.329		1.329	1	1
BCAM1250 (gi77964942)	acetyl-CoA hydrolase/transferase [*Burkholderia* sp. 383]											
BCAM1291	L-asparaginase											
BCAM1304	conserved hypothetical protein											
BCAM1351	putative regulatory protein		1.887	1.887		0.963	0.963		0.825	0.825	1	0
BCAM1380 (gi118650657)	transcriptional regulator, GntR family [*Burkholderia cenocepacia* MC0-3]	1.935	1.901	1.918	1.143	1.275	1.207	0.848	1.053	0.945	1	0
BCAM1407	DJ-1/PfpI family protein	1.58	1.219	1.388	0.967	0.632	0.782	0.788	0.694	0.740	0	0
BCAM1411	putative short-chain dehydrogenase											
BCAM1412 (gi105895814)	conserved hypothetical protein [*Burkholderia cenocepacia* AU 1054]											
BCAM1443	putative exported protein	2.306		2.306	1.093		1.093	1.064		1.064	1	0
BCAM1464 (gi116650430)	putative cytoplasmic protein [*Burkholderia cenocepacia* HI2424]	0.35	0.565	0.445	0.616	0.629	0.622	0.798	1.276	1.009	-1	-1
BCAM1481	conserved hypothetical protein	1.408	1.717	1.555	0.864	1.053	0.954	0.859	0.983	0.919	1	0
BCAM1491 (gi116650457)	conserved hypothetical protein [*Burkholderia cenocepacia* HI2424]											
BCAM1538	putative dehydrogenase, monooxygenase subunit	7.769	7.287	7.524	2.23	3.182	2.664	3.306	3.379	3.342	1	1
BCAM1542	putative aldehyde dehydrogenase	0.595		0.595	1.31		1.310	1.608		1.608	-1	0
BCAM1553	putative dehydrogenase, molybdenum-binding											
BCAM1570	alcohol dehydrogenase	0.53	0.666	0.594	0.919	0.943	0.931	0.918	0.981	0.949	-1	0
BCAM1581	phosphoenolpyruvate carboxykinase [GTP]	0.835		0.835	1.669		1.669	1.123		1.123	0	1
BCAM1619	putative DNA-binding cold-shock protein	0.818	0.775	0.796	0.867	0.805	0.835	0.793	0.733	0.762	0	0
BCAM1654 (gi77969268)	Short-chain dehydrogenase/reductase SDR [*Burkholderia* sp. 383]	0.668	0.778	0.721	0.639	0.689	0.664	0.441	0.511	0.475	0	-1
BCAM1669	putative exported protein											
BCAM1679 (gi117985553)	protein of unknown function DUF472 [*Burkholderia phymatum* STM815]	2.446		2.446	1.25		1.250	1.507		1.507	1	0
BCAM1700 (gi117993315)	GCN5-related N-acetyltransferase [*Burkholderia phytofirmans* PsJN]											
BCAM1744	serine peptidase, family S9	0.661	0.634	0.647	1.275	0.838	1.034	0.919	0.716	0.811	-1	0
BCAM1800	conserved hypothetical protein											
BCAM1824	conserved hypothetical protein											
BCAM1857	conserved hypothetical protein	0.36		0.360	0.847		0.847	1.004		1.004	-1	0
BCAM1919 (gi118648785)	conserved hypothetical protein [*Burkholderia cenocepacia* MC0-3]											

BCAM1926	conserved hypothetical protein											
BCAM1931	putative porin	0.369	0.616	0.477	0.928	1.078	1.000	1.043	1.35	1.187	-1	0
BCAM2001 (gi118650319)	short-chain dehydrogenase/reductase SDR [*Burkholderia cenocepacia* MC0-3]											
BCAM2006	putative aspartate carbomyltransferase											
BCAM2068	conserved hypothetical protein											
BCAM2081	conserved hypothetical protein	1.265		1.265	0.775		0.775	0.656		0.656	0	0
BCAM2143	cable pilus associated adhesin protein	0.131	0.229	0.173	0.222	0.17	0.194	0.272	0.499	0.368	-1	-1
BCAM2159	putative exported protein	2.165	2.018	2.090	0.821	0.77	0.795	0.864	0.86	0.862	1	0
BCAM2167	conserved hypothetical protein	3.645		3.645	1.188		1.188	1.045		1.045	1	0
BCAM2192	enoyl-CoA hydratase/isomerase family protein											
BCAM2195	putative AMP-binding enzyme											
BCAM2234 (gi76582946)	salicylate biosynthesis protein [*Burkholderia pseudomallei* 1710b]											
BCAM2307	zinc metalloprotease ZmpB	0.272	0.446	0.348	0.197	0.285	0.237	0.71	0.906	0.802	-1	-1
BCAM2308	putative leucyl aminopeptidase precursor	0.493		0.493	0.527		0.527	0.661		0.661	-1	-1
BCAM2310 (gi52422765)	putative transporter [*Burkholderia mallei* ATCC 23344]											
BCAM2378	putative Xaa-Pro dipeptidyl-peptidase	0.443	0.417	0.430	0.624	0.582	0.603	1.085	1.329	1.201	-1	-1
BCAM2407 (gi117982799)	major facilitator superfamily MFS_1 [*Burkholderia phymatum* STM815]	1.707		1.707	0.963		0.963	1.121		1.121	1	0
BCAM2456 (gi118660218)	peptidoglycan glycosyltransferase [*Burkholderia multivorans* ATCC 17616]											
BCAM2463	putative membrane protein	0.654		0.654	1.361		1.361	0.948		0.948	-1	0
BCAM2468	putative aldehyde dehydrogenase family protein		0.607	0.607		0.826	0.826		0.823	0.823	-1	0
BCAM2478	serine-carboxyl peptidase											
BCAM2480 (gi52423083)	transcriptional regulator, AsnC family [*Burkholderia mallei* ATCC 23344]	1.888	2.261	2.066	1.209	1.278	1.243	0.931	1.065	0.996	1	0
BCAM2556	putative purine nucleoside permease	0.398	0.839	0.578	0.814	0.865	0.839	1.027	0.937	0.981	-1	0
BCAM2618	putative periplasmic	2.085	2.125	2.105	0.933	1.165	1.043	0.996	1.014	1.005	1	0
BCAM2671	putative low-specificity L-threonine aldolase		1.11	1.110		1.515	1.515		1.901	1.901	0	1
BCAM2702	2-methylcitrate synthase	0.334	1.166	0.624	0.759	1.303	0.994	0.567	1.54	0.934	-1	0
BCAM2707	putative FAA-hydrolase family protein											
BCAM2755	putative exported protein	2.116	2.476	2.289	0.716	0.866	0.787	0.93	1.08	1.002	1	0
BCAM2783 (gi117988745)	glycoside hydrolase, family 28 [*Burkholderia phytofirmans* PsJN]											
BCAM2821	malate synthase G											
BCAM2827	putative exported protein	1.917	1.696	1.803	1.246	1.216	1.231	1.347	1.001	1.161	1	0
BCAS0010 (gi77965393)	protein of unknown function DUF6, transmembrane [*Burkholderia* sp. 383]	2.487	1.889	2.167	1.394	1.053	1.212	1.148	1.094	1.121	1	0
BCAS0013	putative molybdenum transport protein											

BCAS0070 (gi118660709)	histidine kinase [*Burkholderia multivorans* ATCC 17616]	4.169		4.169	1.043		1.043	1.135		1.135	1	0
BCAS0151	hypothetical protein	0.417	0.438	0.427	0.656	0.665	0.660	0.467	0.369	0.415	-1	-1
BCAS0190	putative H-NS family DNA-binding protein	4.788	3.732	4.227	1.209	1.224	1.216	1.156	0.938	1.041	1	0
BCAS0292	conserved hypothetical protein AidA'	0.246		0.246	0.434		0.434	0.986		0.986	-1	-1
BCAS0293	nematocidal protein AidA	0.08	0.339	0.165	0.169	0.364	0.248	1.022	1.3	1.153	-1	-1
BCAS0334 (gi134132307)	response regulator receiver protein [*Burkholderia vietnamiensis* G4]		1.96	1.960		1.024	1.024		1.307	1.307	1	0
BCAS0382 (gi117994159)	transcriptional regulator, AsnC family [*Burkholderia phytofirmans* PsJN]											
BCAS0407	hypothetical protein											
BCAS0409	zinc metalloprotease ZmpA	0.459	0.552	0.503	0.454	0.447	0.450	0.683	0.826	0.751	-1	-1
BCAS0421 (gi76580611)	ABC transporter, ATP-binding protein [*Burkholderia pseudomallei* 1710b]		1.918	1.918		1.1	1.100		0.835	0.835	1	0
BCAS0455	putative CopG family protein											
BCAS0516 (gi115281897)	hypothetical protein [*Burkholderia cepacia* AMMD]	1.304	1.886	1.568	0.796	1.054	0.916	0.616	0.666	0.641	1	0
BCAS0609	putative electron transfer											
BCAS0637	60 kDa chaperonin 3	1.83	1.706	1.767	1.485	1.277	1.377	1.549	1.266	1.400	1	0
BCAS0638	10 kDa chaperonin 3	1.368	1.099	1.226	0.628	0.832	0.723	0.92	0.835	0.876	0	0
BCAS0666	putative ankyrin-repeat exported protein											
BCAS0708 (gi91686888)	hypothetical protein [*Burkholderia xenovorans* LB400]											
BCAS0730 (gi77964328)	Na+ dependent nucleoside transporter [*Burkholderia* sp. 383]											
BCAS0739	putative acetyl-CoA synthetase	0.534		0.534	1.3		1.300	1.058		1.058	-1	0
BCAS0747 (gi117980068)	SMC protein-like [*Burkholderia phymatum* STM815]											
BCAS0765 (gi77969929)	hydrophobe/amphiphile efflux pump, HAE1 family [*Burkholderia* sp. 383]		2.226	2.226		1.993	1.993		1.545	1.545	1	1
BCAS0773	putative exported protein	2.771		2.771	0.691		0.691	1.491		1.491	1	0
rmlC (gi52210708)	dTDP-6-deoxy-D-glucose-3,5 epimerase [*Burkholderia pseudomallei* K96243]											
wbiF (gi134134232)	glycosyl transferase, family 2 [*Burkholderia vietnamiensis* G4]	0.951		0.951	1.671		1.671	0.884		0.884	0	1
xdhA (gi115283038)	transcriptional regulator, LysR family [*Burkholderia cepacia* AMMD]	2.679		2.679	1.465		1.465	1.28		1.280	1	0

Whole cell proteins

Accession No.	Protein Name	Avg. iTRAQ Ratio 116/117* a	115/114* b	WC cepR / WT normalized	Avg. iTRAQ Ratio 115/117* a	116/114* b	WC cepI / wT normalized	Avg. iTRAQ Ratio 114/117* a	117/114* b	WC cepI+C8-HSL / WT normalized	Threshold reached ≥1.5 => up-regulated (1) ≤0.67=> down-regulated (-1) WC cepR	WC cepI
BCAL0009	pterin-4-alpha-carbinolamine dehydratase	1.187	1.298	1.241	1.471	1.489	1.480	0.866	1.198	1.019	0	0
BCAL0012	putative adenylate cyclase	1.058	0.82	0.931	1.569	0.998	1.251	0.968	0.733	0.842	0	0
BCAL0032	ATP synthase B chain	1.14	0.808	0.960	0.788	0.506	0.631	1.006	0.855	0.927	0	-1
BCAL0036 (gi105894680)	ATP synthase F1, beta subunit [*Burkholderia cenocepacia* AU 1054]	0.882	0.889	0.885	0.861	0.641	0.743	0.942	1.152	1.042	0	0
BCAL0037	ATP synthase epsilon chain											
BCAL0038 (gi117992142)	AMP-dependent synthetase and ligase [*Burkholderia phytofirmans* PsJN]											
BCAL0043	putative extracellular ligand-binding protein	1.311	1.394	1.352	1.26	1.188	1.223	1.113	1.012	1.061	0	0
BCAL0052 (gi117984267)	FAD linked oxidase-like [*Burkholderia phymatum* STM815]	1.391		1.391	1.587		1.587	1.077		1.077	0	1
BCAL0074	glycine cleavage system H protein	1.366	0.98	1.157	1.931	1.339	1.608	0.909	1.064	0.983	0	1
BCAL0077	putative oxidoreductase		0.733	0.733		0.596	0.596		1.114	1.114	0	-1
BCAL0080	putative cytochrome		1.362	1.362		0.513	0.513		1.207	1.207	0	-1
BCAL0095	putative phage major tail sheath protein											
BCAL0114 (gi105894597)	flagellin-like [*Burkholderia cenocepacia* AU 1054]											
BCAL0115	30S ribosomal protein S21 1	0.959	1.194	1.070	0.777	0.998	0.881	0.762	0.883	0.820	0	0
BCAL0122	histone-like nucleoid-structuring (H-NS)	0.413	0.476	0.443	0.416	0.328	0.369	0.44	0.446	0.443	-1	-1
BCAL0154	histone-like nucleoid-structuring (H-NS)											
BCAL0202	putative flavoprotein	1.452	1.058	1.239	3.559	1.202	2.068	1.26	0.925	1.080	0	1
BCAL0210	TetR family regulatory protein	1.636	1.28	1.447	1.802	1.629	1.713	1.497	1.506	1.501	0	1
BCAL0215	phenylacetic acid degradation protein PaaB	0.946	0.883	0.914	0.991	0.868	0.927	0.887	0.955	0.920	0	0
BCAL0216	phenylacetic acid degradation protein PaaA	0.974		0.974	0.986		0.986	1.313		1.313	0	0
BCAL0217 (gi52427635)	lipase/acylhydrolase, putative [*Burkholderia mallei* ATCC 23344]											
BCAL0221	transcription antitermination protein NusG	1.279	1.19	1.234	1.504	1.063	1.264	0.975	0.984	0.979	0	0
BCAL0224	50S ribosomal protein L10	1.013	0.906	0.958	1.002	0.64	0.801	0.906	0.777	0.839	0	0

BCAL0231	elongation factor G	0.866	0.781	0.822	0.826	0.705	0.763	1.054	0.943	0.997	0	0
BCAL0236	50S ribosomal protein L23	1.258	0.981	1.111	1.365	0.953	1.141	0.997	0.783	0.884	0	0
BCAL0237	50S ribosomal protein L2	1.069	1.168	1.117	0.984	1.022	1.003	0.787	0.695	0.740	0	0
BCAL0238	30S ribosomal protein S19	0.821	0.889	0.854	0.696	0.745	0.720	1.057	1.02	1.038	0	0
BCAL0239	50S ribosomal protein L22	1.243	1.952	1.558	1.317	1.509	1.410	0.977	1.128	1.050	1	0
BCAL0240	30S ribosomal protein S3	0.779	1.082	0.918	0.594	0.707	0.648	0.706	0.958	0.822	0	-1
BCAL0241	50S ribosomal protein L16	1.269	1.342	1.305	0.851	0.61	0.720	0.763	0.924	0.840	0	0
BCAL0243	30S ribosomal protein S17	0.915	0.851	0.882	0.739	0.838	0.787	0.807	0.835	0.821	0	0
BCAL0245	50S ribosomal protein L24	1.046	1.09	1.068	0.817	1.182	0.983	0.803	1.015	0.903	0	0
BCAL0247	30S ribosomal protein S14		2.003	2.003		0.787	0.787		1.127	1.127	1	0
BCAL0249	50S ribosomal protein L6	1.075	0.968	1.020	1.218	0.899	1.046	0.804	0.741	0.772	0	0
BCAL0251	30S ribosomal protein S5	0.993	1.11	1.050	0.872	1.023	0.944	0.959	0.96	0.959	0	0
BCAL0253	50S ribosomal protein L15	1.182	0.974	1.073	1.401	0.856	1.095	0.775	0.743	0.759	0	0
BCAL0255	translation initiation factor IF-1	0.869	1.054	0.957	0.979	2.117	1.440	1.105	1.301	1.199	0	0
BCAL0258	30S ribosomal protein S11	0.928		0.928	0.907		0.907	0.818		0.818	0	0
BCAL0264	delta-aminolevulinic acid dehydratase	1.159	1.151	1.155	1.569	1.238	1.394	1.063	0.951	1.005	0	0
BCAL0273	protein CyaY	1.019	1.305	1.153	1.075	1.637	1.327	0.993	1.459	1.204	0	0
BCAL0327	conserved hypothetical protein	1.27	1.214	1.242	1.932	1.323	1.599	1.143	0.994	1.066	0	1
BCAL0332	putative stringent starvation protein B		1.078	1.078		1.036	1.036		0.72	0.720	0	0
BCAL0334	periplasmic solute-binding protein		1.126	1.126		2.371	2.371		1.171	1.171	0	1
BCAL0341O	conserved hypothetical protein	0.635	0.831	0.726	0.74	0.886	0.810	0.857	1.321	1.064	0	0
BCAL0342	conserved hypothetical protein	0.654	0.671	0.662	0.539	1.175	0.796	1.063	1.628	1.316	-1	0
BCAL0343	conserved hypothetical protein 3	0.896	1.018	0.955	0.771	0.866	0.817	0.93	1.047	0.987	0	0
BCAL0347	protease associated ATPase ClpB	0.45		0.450	0.386		0.386	0.795		0.795	-1	-1
BCAL0349	putative outer membrane protein		1.932	1.932		1.477	1.477		1.583	1.583	1	0
BCAL0353	putative membrane protein	0.54		0.540	0.687		0.687	0.946		0.946	-1	0
BCAL0358	metallo peptidase, family M1											
BCAL0360	conserved hypothetical protein	0.72	0.322	0.481	0.951	0.872	0.911	0.859	0.945	0.901	-1	0
BCAL0371	putative aromatic acid decarboxylase	1.635		1.635	1.325		1.325	1.55		1.550	1	0
BCAL0387	putative GTP-binding protein	0.773	0.953	0.858	0.831	0.523	0.659	1.137	0.977	1.054	0	-1
BCAL0426	putative membrane protein		0.71	0.710		0.638	0.638		0.583	0.583	0	-1
BCAL0482	putative rod shape-determining protein	0.752	0.644	0.696	0.769	0.212	0.404	0.932	0.539	0.709	0	-1
BCAL0487	endonuclease/exonuclease/phosphatase family											
BCAL0502	DksA/TraR C4-type zinc finger family protein	0.967	1.021	0.994	1.241	1.033	1.132	0.966	0.888	0.926	0	0
BCAL0503	putative cobalamin synthesis protein	0.891		0.891	0.756		0.756	1.111		1.111	0	0
BCAL0507	diaminopimelate epimerase		0.679	0.679		0.576	0.576		0.917	0.917	0	-1

BCAL0529	conserved hypothetical protein		1.142	1.142		1.553	1.553		2.132	2.132	0	1
BCAL0536	ferredoxin--NADP reductase	0.931	0.922	0.926	0.866	1.147	0.997	0.998	1.086	1.041	0	0
BCAL0557	putative glutathione S-transferase protein		0.681	0.681		0.57	0.570		0.874	0.874	0	-1
BCAL0562	negative regulator of flagellin synthesis		1.888	1.888		1.665	1.665		1.435	1.435	1	1
BCAL0565	flagellar basal-body rod protein FlgC											
BCAL0567	flagellar hook protein 1 FlgE1	1.249		1.249	1.219		1.219	1.329		1.329	0	0
BCAL0568	flagellar basal-body rod protein FlgF											
BCAL0569	flagellar basal-body rod protein FlgG		1.599	1.599		1.453	1.453		1.697	1.697	1	0
BCAL0576	flagellar hook-associated protein 1 (HAP1)											
BCAL0577	flagellar hook-associated protein 3 (HAP3)		0.882	0.882		1.061	1.061		1.066	1.066	0	0
BCAL0616 (gi83653191)	uncharacterized ACR, COG1565 superfamily [*Burkholderia thailandensis* E264]	0.849		0.849	0.567		0.567	0.813		0.813	0	-1
BCAL0617	conserved hypothetical protein	1.861		1.861	0.945		0.945	1.391		1.391	1	0
BCAL0626	putative 2-nitropropane dioxygenase		0.719	0.719		0.638	0.638		0.674	0.674	0	-1
BCAL0650	putative pyruvate-flavodoxin oxidoreductase											
BCAL0658	allophanate hydrolase subunit 2		1.524	1.524		1.255	1.255		1.029	1.029	1	0
BCAL0677	thiol:disulfide interchange protein 7	0.799	0.824	0.811	0.888	0.778	0.831	0.979	0.807	0.889	0	0
BCAL0680	conserved hypothetical protein	0.972	0.909	0.940	1.961	0.919	1.342	1.09	0.571	0.789	0	0
BCAL0690	conserved hypothetical protein	2.348		2.348	2.669		2.669	1.831		1.831	1	1
BCAL0703	serine peptidase, family S9		0.845	0.845		1.581	1.581		1.401	1.401	0	1
BCAL0704	D-alanyl-D-alanine carboxypeptidase		0.49	0.490		0.694	0.694		0.846	0.846	-1	0
BCAL0728	conserved hypothetical protein	0.958	0.998	0.978	0.818	0.792	0.805	1.068	0.761	0.902	0	0
BCAL0738	C-terminal processing protease-3	0.897	0.802	0.848	1.123	0.993	1.056	1.213	1.161	1.187	0	0
BCAL0742	protein-export protein	0.935	1.187	1.053	1.029	0.524	0.734	1.15	0.794	0.956	0	0
BCAL0763	putative exported protein	0.998	1.069	1.033	1.138	1.126	1.132	0.974	1.101	1.036	0	0
BCAL0764	putative exported protein		1	1.000		1.518	1.518		1.274	1.274	0	1
BCAL0783	putative membrane protein		1.583	1.583		0.814	0.814		0.903	0.903	1	0
BCAL0788	putative exported protein	0.928	1.023	0.974	1.431	1.375	1.403	1.219	1.486	1.346	0	0
BCAL0794	conserved hypothetical protein		1.035	1.035		1.593	1.593		1.393	1.393	0	1
BCAL0799	ribosomal L25p family protein	0.924	1.169	1.039	1.15	0.933	1.036	0.754	0.654	0.702	0	0
BCAL0800	ribose-phosphate pyrophosphokinase	0.556	1.311	0.854	0.4	0.82	0.573	0.772	1.104	0.923	0	-1
BCAL0804	putative membrane protein	0.951	1.035	0.992	1.077	1.035	1.056	1.199	1.22	1.209	0	0
BCAL0812	sigma-54 modulation protein	1.373	1.194	1.280	1.461	1.418	1.439	1.181	0.988	1.080	0	0
BCAL0817	putative 3-deoxy-D-manno-octulosonate		1.743	1.743		1.458	1.458		0.962	0.962	1	0
BCAL0825	excinuclease ABC subunit A	0.813		0.813	0.594		0.594	1.124		1.124	0	-1
BCAL0831	putative storage protein		0.284	0.284		0.432	0.432		0.423	0.423	-1	-1
BCAL0849	metallo peptidase, subfamily M48B	0.637	0.587	0.611	0.495	0.577	0.534	0.485	0.316	0.391	-1	-1

BCAL0874	putative membrane protein	1.192	0.893	1.032	1.189	1.024	1.103	0.957	1.036	0.996	0	0
BCAL0878	conserved hypothetical protein	1.09	1.254	1.169	1.362	1.383	1.372	0.987	1.181	1.080	0	0
BCAL0895	putative peptidyl-prolyl cis-trans isomerase	1.064	1.096	1.080	1.264	1.236	1.250	1.121	1.068	1.094	0	0
BCAL0926	putative glycerol-3-phosphate dehydrogenase	0.665		0.665	0.598		0.598	0.915		0.915	-1	-1
BCAL0934	putative periplasmic cytochrome c containing		0.974	0.974		1.13	1.130		1.235	1.235	0	0
BCAL0935	putative periplasmic cytochrome c protein	1.431	0.898	1.134	2.577	0.667	1.311	1.389	0.956	1.152	0	0
BCAL0965	putative hydrolase 1		0.62	0.620		1.205	1.205		0.88	0.880	-1	0
BCAL0980 (gi83653798)	molybdopterin biosynthesis moeA protein [*Burkholderia thailandensis* E264]											
BCAL1044	GntR family regulatory protein											
BCAL1065	periplasmic solute-binding protein	0.958	1.11	1.031	1.317	0.996	1.145	0.873	0.844	0.858	0	0
BCAL1072	conserved hypothetical protein		1.025	1.025		1.153	1.153		1.076	1.076	0	0
BCAL1092	ABC transporter extracellular solute-binding	0.92	0.698	0.801	1.822	0.802	1.209	0.994	0.925	0.959	0	0
BCAL1215	dihydrolipoamide dehydrogenase		1.763	1.763		0.73	0.730		1.149	1.149	1	0
BCAL1216 (gi116647358)	male sterility C-terminal domain [*Burkholderia cenocepacia* HI2424]											
BCAL1259 (gi117988480)	hypothetical protein [*Burkholderia phytofirmans* PsJN]											
BCAL1262	carbamoyl-phosphate synthase large chain											
BCAL1263	transcription elongation factor	0.96	1.205	1.076	0.884	1.061	0.968	0.79	0.867	0.828	0	0
BCAL1394	putative exported protein		1.253	1.253		1.654	1.654		1.558	1.558	0	1
BCAL1411	putative exported protein	0.965	1.075	1.019	0.878	1.135	0.998	0.857	1.083	0.963	0	0
BCAL1413	glutaminyl-tRNA synthetase		1.294	1.294		1.446	1.446		1.292	1.292	0	0
BCAL1416	alanyl-tRNA synthetase	0.867	0.393	0.584	0.951	0.825	0.886	1.46	1.43	1.445	-1	0
BCAL1472	succinyl-CoA:3-ketoacid-coenzyme A transferase	1.136	0.945	1.036	2.051	1.386	1.686	1.638	1.917	1.772	0	1
BCAL1478	putative hydrolase		0.991	0.991		0.608	0.608		0.673	0.673	0	-1
BCAL1482	translation initiation factor IF-3	0.932	1.349	1.121	0.807	1.024	0.909	0.863	1.193	1.015	0	0
BCAL1484	50S ribosomal protein L20	1.122	2.041	1.513	0.967	1.624	1.253	0.77	1.598	1.109	1	0
BCAL1486	phenylalanyl-tRNA synthetase beta chain		1.321	1.321		0.544	0.544		1.308	1.308	0	-1
BCAL1496	putative exported protein	1.169	1.011	1.087	2.119	0.539	1.069	0.808	0.285	0.480	0	0
BCAL1497 (gi91686933)	putative lipoprotein transmembrane [*Burkholderia xenovorans* LB400]											
BCAL1501 (gi117993520)	transcriptional regulator, LysR family [*Burkholderia phytofirmans* PsJN]		0.656	0.656		1.04	1.040		0.846	0.846	-1	0
BCAL1504	RNA pseudouridylate synthase family protein	0.857	0.953	0.904	0.848	1.208	1.012	1.299	0.984	1.131	0	0
BCAL1506	N utilization substance protein A	0.958	0.956	0.957	0.854	1.033	0.939	1.038	0.838	0.933	0	0
BCAL1507	translation initiation factor IF-2	0.832	0.956	0.892	0.868	1.08	0.968	1.077	1.269	1.169	0	0
BCAL1515	2-oxoglutarate dehydrogenase E1 component	1.1	2.88	1.780	0.867	1.601	1.178	1.294	1.398	1.345	1	0
BCAL1530	flp pilus type assembly protein		1.516	1.516		0.827	0.827		1.351	1.351	1	0

BCAL1611	dihydroorotate dehydrogenase		1.424	1.424		1.521	1.521		1.104	1.104	0	1
BCAL1636	putative exported endonuclease		0.886	0.886		0.63	0.630		1.346	1.346	0	-1
BCAL1657	putative ribose transport system	1.292	1.024	1.150	1.684	0.971	1.279	0.923	0.702	0.805	0	0
BCAL1663	PrkA family serine protein kinase	0.926	0.705	0.808	0.58	0.522	0.550	1.233	1.026	1.125	0	-1
BCAL1675 (gi76580894)	TetR family regulatory protein [*Burkholderia pseudomallei* 1710b]											
BCAL1677	putative type-1 fimbrial protein											
BCAL1696 (gi83650419)	nonribosomal peptide synthetase, putative [*Burkholderia thailandensis* E264]	1.114		1.114	0.633		0.633	1.194		1.194	0	-1
BCAL1722 (gi116647781)	chitinase [*Burkholderia cenocepacia* HI2424]											
BCAL1824	putrescine-binding periplasmic protein	0.855	0.963	0.907	1.054	0.994	1.024	1.173	1.246	1.209	0	0
BCAL1831	putative betaine aldehyde dehydrogenase	1.388	1.167	1.273	1.145	1.056	1.100	0.904	1.279	1.075	0	0
BCAL1849	putative exported protein	0.608	0.906	0.742	0.575	0.431	0.498	0.86	0.767	0.812	0	-1
BCAL1850	putative dehydrogenase		0.524	0.524		0.67	0.670		1.281	1.281	-1	0
BCAL1868	conserved hypothetical protein	1.188	1.13	1.159	1.531	1.073	1.282	0.823	0.79	0.806	0	0
BCAL1869	putative exported protein		4.543	4.543		1.779	1.779		2.858	2.858	1	1
BCAL1872	putative nucleotide phosphoribosyltransferase		0.753	0.753		0.645	0.645		0.543	0.543	0	-1
BCAL1874	putative ATP phosphoribosyltransferase		0.601	0.601		0.899	0.899		0.8	0.800	-1	0
BCAL1879	Hfq protein	0.731	1.005	0.857	0.707	0.922	0.807	0.862	0.959	0.909	0	0
BCAL1892	RNA polymerase sigma factor	1.083	0.91	0.993	1.569	1.169	1.354	0.876	1.232	1.039	0	0
BCAL1898	conserved hypothetical protein		0.656	0.656		0.774	0.774		1.215	1.215	-1	0
BCAL1900	thioredoxin	0.991	0.97	0.980	1.087	1.344	1.209	1.223	1.175	1.199	0	0
BCAL1905	50S ribosomal protein L31	1.204	1.267	1.235	1.096	1.172	1.133	1.111	1.079	1.095	0	0
BCAL1917	putative exported protein	0.815	0.879	0.846	0.817	1.086	0.942	0.747	1.102	0.907	0	0
BCAL1919 (gi118654245)	ATPase AAA-2 [*Burkholderia cenocepacia* MC0-3]		0.592	0.592		0.711	0.711		1.234	1.234	-1	0
BCAL1934	UDP-glucuronic acid decarboxylase		0.871	0.871		0.579	0.579		0.569	0.569	0	-1
BCAL1942	50S ribosomal protein L9	1.182	1.211	1.196	1.323	1.084	1.198	0.926	0.883	0.904	0	0
BCAL1943	30S ribosomal protein S18	0.965		0.965	0.721		0.721	0.806		0.806	0	0
BCAL1945	putative 30S ribosomal protein S6	1.181	1.277	1.228	0.999	1.081	1.039	1.319	0.563	0.862	0	0
BCAL1946 (gi52427005)	conserved hypothetical protein [*Burkholderia mallei* ATCC 23344]	0.722		0.722	0.3		0.300	1.151		1.151	0	-1
BCAL1956	putative lipoprotein	0.806		0.806	0.73		0.730	0.883		0.883	0	0
BCAL1961 (gi105898095)	ankyrin repeat protein [*Burkholderia cenocepacia* AU 1054]		0.616	0.616		0.642	0.642		0.652	0.652	-1	-1
BCAL1964	putative thymidylate kinase	0.691	0.832	0.758	0.616	0.665	0.640	0.902	0.605	0.739	0	-1
BCAL1971	conserved hypothetical protein		0.653	0.653		0.495	0.495		0.865	0.865	-1	-1
BCAL1982	peptide methionine sulfoxide reductase		0.602	0.602		0.866	0.866		0.926	0.926	-1	0
BCAL1985	putative exported isomerase	1.212	1.062	1.135	1.474	1.192	1.326	1.253	1.06	1.152	0	0

BCAL1992 (gi77967434)	lipolytic enzyme, G-D-S-L [*Burkholderia* sp. 383]	1.447		1.447	1.11		1.110	1.188		1.188	0	0
BCAL1995	ATP-dependent Clp protease ATP-binding subunit	1.816	0.831	1.228	2.597	0.867	1.501	1.279	1.116	1.195	0	1
BCAL1996	ATP-dependent Clp protease proteolytic subunit	1.087	0.844	0.958	1.328	0.972	1.136	1.009	0.713	0.848	0	0
BCAL1997	trigger factor	1.11	0.999	1.053	1.352	0.94	1.127	0.898	0.906	0.902	0	0
BCAL1998	putative kinase	0.891	1.924	1.309	1.345	2.199	1.720	1.37	1.951	1.635	0	1
BCAL2013	AhpC/TSA family protein	0.7	0.692	0.696	0.628	0.685	0.656	0.871	0.856	0.863	0	-1
BCAL2018 (gi105898280)	transcriptional regulator, GntR family [*Burkholderia cenocepacia* AU 1054]	0.944		0.944	0.83		0.830	0.867		0.867	0	0
BCAL2022	PspA/IM30 family protein	0.872	1.067	0.965	0.873	0.899	0.886	0.914	0.927	0.920	0	0
BCAL2039	putative uricase		0.898	0.898		0.503	0.503		0.703	0.703	0	-1
BCAL2063	inosine-5'-monophosphate dehydrogenase	1.126	1.291	1.206	1.289	0.888	1.070	1.056	1.146	1.100	0	0
BCAL2082	chaperone protein Skp precursor	1.033	1.042	1.037	1.166	1.149	1.157	1.157	1.061	1.108	0	0
BCAL2083 (gi118657256)	surface antigen (D15) [*Burkholderia multivorans* ATCC 17616]											
BCAL2088	ribosome recycling factor		1.176	1.176		1.201	1.201		1.016	1.016	0	0
BCAL2091	30S ribosomal protein S2	1.14	0.884	1.004	1.036	0.919	0.976	0.995	0.934	0.964	0	0
BCAL2094 (gi91688077)	pseudouridine synthase, Rsu [*Burkholderia xenovorans* LB400]											
BCAL2098	putative chromosome partition protein											
BCAL2110 (gi52427334)	alanine racemase [*Burkholderia mallei* ATCC 23344]	1.534		1.534	1.387		1.387	0.711		0.711	1	0
BCAL2123	conserved hypothetical protein		1.84	1.840		1.622	1.622		1.489	1.489	1	1
BCAL2126	glutamyl-tRNA synthetase		0.789	0.789		0.548	0.548		1.04	1.040	0	-1
BCAL2150	cysteinyl-tRNA synthetase	1.437	0.973	1.182	2.714	0.988	1.638	1.549	1.209	1.368	0	1
BCAL2152	peptidyl-prolyl cis-trans isomerase A precursor	1.213	1.079	1.144	1.797	1.405	1.589	1.193	1.22	1.206	0	1
BCAL2166	putative lipoprotein	1.361		1.361	1.146		1.146	1.437		1.437	0	0
BCAL2180	putative 2-dehydro-3-deoxyphosphooctonate	1.891	1.983	1.936	1.177	1.394	1.281	0.81	1.006	0.903	1	0
BCAL2189	peptide chain release factor 2	1.308	0.8	1.023	1.146	0.79	0.951	1.155	1.009	1.080	0	0
BCAL2190 (gi117980152)	lysyl-tRNA synthetase [*Burkholderia phymatum* STM815]	1.598	1.468	1.532	1.353	1.028	1.179	2.135	1.009	1.468	1	0
BCAL2206	phasin-like protein	0.953	1.029	0.990	1.141	1.136	1.138	1.251	1.588	1.409	0	0
BCAL2209	pyruvate dehydrogenase E1 component	0.821	0.893	0.856	0.633	0.547	0.588	0.796	0.756	0.776	0	-1
BCAL2211	two-component regulatory system, response	0.917		0.917	0.914		0.914	1.127		1.127	0	0
BCAL2226	putative molybdopterin-binding protein		0.425	0.425		0.55	0.550		2.222	2.222	-1	-1
BCAL2288	bacterioferritin	1.186	1.106	1.145	1.278	1.019	1.141	0.968	0.95	0.959	0	0
BCAL2300	putative exported protein	1.392	1.019	1.191	1.006	1.457	1.211	1.209	1.145	1.177	0	0
BCAL2329	NUDIX hydrolase	1.389		1.389	1.557		1.557	1.498		1.498	0	1
BCAL2336	putative NADH dehydrogenase I chain I											

BCAL2340	putative NADH dehydrogenase I chain E	0.865	1.858	1.268	0.936	0.275	0.507	1.012	2.929	1.722	0	-1
BCAL2342	NADH dehydrogenase I chain C											
BCAL2349	30S ribosomal protein S15	1.299	1.028	1.156	1.367	0.947	1.138	0.827	0.707	0.765	0	0
BCAL2354	2-isopropylmalate synthase	0.478	0.467	0.472	0.698	0.486	0.582	1.069	0.673	0.848	-1	-1
BCAL2359	acetolactate synthase isozyme III large subunit	0.663		0.663	0.667		0.667	0.772		0.772	-1	-1
BCAL2384	quinone oxidoreductase	1.169	1.058	1.112	1.795	1.343	1.553	1.323	1.359	1.341	0	1
BCAL2388	conserved hypothetical protein	0.913	1.562	1.194	0.871	2.277	1.408	0.988	2.094	1.438	0	0
BCAL2394 (gi52428872)	maf protein [*Burkholderia mallei* ATCC 23344]	0.707	0.834	0.768	0.556	0.723	0.634	1.093	1.325	1.203	0	-1
BCAL2396	conserved hypothetical protein	0.989	1.403	1.178	1.028	1.115	1.071	0.919	1.399	1.134	0	0
BCAL2401	putative exported protein											
BCAL2409 (gi121229043)	DNA polymerase III, alpha subunit, form 1 [*Burkholderia mallei* SAVP1]											
BCAL2415	phosphoribosylglycinamide formyltransferase 2		0.647	0.647		0.815	0.815		1.139	1.139	-1	0
BCAL2418	putative exported protein		0.935	0.935		1.266	1.266		1.353	1.353	0	0
BCAL2429	putative cytochrome C precursor-related protein	0.655	1.016	0.816	0.821	0.791	0.806	1.109	0.882	0.989	0	0
BCAL2446	putative aminotransferase		0.734	0.734		0.468	0.468		1.354	1.354	0	-1
BCAL2476a	conserved hypothetical protein (fragment)											
BCAL2606	two-component regulatory system, response	0.958	1.196	1.070	0.956	1.013	0.984	0.777	1.291	1.002	0	0
BCAL2607	putative exported protein	0.882	0.932	0.907	0.933	1.052	0.991	0.733	0.628	0.678	0	0
BCAL2616	nitrogen regulatory protein P-II 2	1.264	1.143	1.202	1.212	1.224	1.218	1.102	0.971	1.034	0	0
BCAL2622	inorganic pyrophosphatase	1.201	0.908	1.044	0.811	0.937	0.872	1.15	0.98	1.062	0	0
BCAL2630	putative porphobilinogen deaminase protein	0.754	0.736	0.745	0.671	0.666	0.668	0.84	0.866	0.853	0	-1
BCAL2649	conserved hypothetical protein	0.977	1.255	1.107	1.01	0.977	0.993	0.975	0.98	0.977	0	0
BCAL2653	ParA family ATPase		0.754	0.754		0.547	0.547		0.422	0.422	0	-1
BCAL2657	putative bifunctional cobalamin biosynthesis		0.809	0.809		1.656	1.656		1.035	1.035	0	1
BCAL2666	conserved hypothetical protein	1.354	1.114	1.228	2.153	1.108	1.545	0.947	0.752	0.844	0	1
BCAL2669	putative exported protein	0.607	0.838	0.713	0.578	0.665	0.620	0.477	0.573	0.523	0	-1
BCAL2676	aminopeptidase A	0.926	1.383	1.132	0.879	0.362	0.564	1.022	0.925	0.972	0	-1
BCAL2700	putative oxidoreductase	0.823	0.63	0.720	0.722	0.557	0.634	0.97	0.895	0.932	0	-1
BCAL2710	4-hydroxy-3-methylbut-2-enyl diphosphate	0.859	0.825	0.842	0.831	0.846	0.838	1.168	0.845	0.993	0	0
BCAL2714	50S ribosomal protein L28		0.795	0.795		0.716	0.716		0.727	0.727	0	0
BCAL2732	cold shock-like protein	1.587	1.16	1.357	2.087	1.416	1.719	0.889	0.911	0.900	0	1
BCAL2733 (gi115282608)	multicopper oxidase, type 3 [*Burkholderia cepacia* AMMD]		1.521	1.521		1.49	1.490		1.115	1.115	1	0
BCAL2736	isocitrate dehydrogenase	1.043	0.826	0.928	0.982	0.77	0.870	1.102	0.916	1.005	0	0
BCAL2754	chromate transport protein [*Burkholderia*											

(gi52429288)	*mallei* ATCC 23344]											
BCAL2757	superoxide dismutase SodB	1.082	0.732	0.890	1.343	0.765	1.014	1.268	0.955	1.100	0	0
BCAL2762	putative adenylate kinase	0.892	0.843	0.867	0.887	0.778	0.831	0.918	0.809	0.862	0	0
BCAL2765	putative 30S ribosomal protein S20	1.058	1.447	1.237	1.568	0.988	1.245	1.028	0.781	0.896	0	0
BCAL2769	conserved hypothetical protein	1.018	1.364	1.178	0.937	1.125	1.027	0.885	1.064	0.970	0	0
BCAL2773 (gi117980128)	phage integrase [*Burkholderia phymatum* STM815]											
BCAL2785	putative peptide methionine sulfoxide reductase	1.042	1.581	1.284	1.348	1.442	1.394	0.962	1.445	1.179	0	0
BCAL2791	putative kynureninase		1.016	1.016		1.115	1.115		1.1	1.100	0	0
BCAL2796 (gi118659676)	aldehyde dehydrogenase [*Burkholderia multivorans* ATCC 17616]	2.015		2.015	3.542		3.542	0.62		0.620	1	1
BCAL2821	RND family efflux system transporter protein											
BCAL2828	putative exported protein	0.769	0.682	0.724	0.867	0.77	0.817	0.724	0.655	0.689	0	0
BCAL2839	fructose-bisphosphate aldolase	0.884	1	0.940	0.711	0.601	0.654	1.339	0.862	1.074	0	-1
BCAL2858	hypothetical protein	1.118	0.902	1.004	1.26	0.798	1.003	0.93	0.729	0.823	0	0
BCAL2864	hypothetical protein		0.511	0.511		0.526	0.526		0.687	0.687	-1	-1
BCAL2888	ribonuclease E 1		0.983	0.983		1.418	1.418		1.304	1.304	0	0
BCAL2911 (gi115281004)	conserved hypothetical protein [*Burkholderia cepacia* AMMD]	0.905		0.905	0.619		0.619	1.395		1.395	0	-1
BCAL2915	dihydrofolate reductase		1.097	1.097		1.703	1.703		1.065	1.065	0	1
BCAL2929 (gi91690051)	L-sorbosone dehydrogenase [*Burkholderia xenovorans* LB400]	0.873		0.873	0.541		0.541	0.878		0.878	0	-1
BCAL2935	electron transfer flavoprotein beta-subunit											
BCAL2941	putative exported transglycosylase		0.458	0.458		0.559	0.559		0.412	0.412	-1	-1
BCAL2943	putative exported protein											
BCAL2950	30S ribosomal protein S1	1.189	1.122	1.155	1.22	0.73	0.944	0.981	0.784	0.877	0	0
BCAL2954 (gi77966373)	chorismate mutase/Prephenate dehydratase [*Burkholderia* sp. 383]		0.628	0.628		0.655	0.655		0.6	0.600	-1	-1
BCAL2958	putative ompA family protein	0.693	0.786	0.738	0.55	0.623	0.585	0.797	0.767	0.782	0	-1
BCAL2961 (gi83650624)	integrase [*Burkholderia thailandensis* E264]											
BCAL2966	conserved hypothetical protein	1.162	1.54	1.338	1.683	2.074	1.868	1.466	1.859	1.651	0	1
BCAL3006	cold shock-like protein		0.824	0.824		0.921	0.921		1.004	1.004	0	0
BCAL3008	putative outer membrane porin protein											
BCAL3024 (gi105892254)	conserved hypothetical protein [*Burkholderia cenocepacia* AU 1054]											
BCAL3036	hypothetical protein		0.658	0.658		0.965	0.965		0.819	0.819	-1	0
BCAL3041	maltose-binding protein	1.115		1.115	1.576		1.576	0.945		0.945	0	1
BCAL3051	riboflavin synthase alpha chain	2.868		2.868	1.681		1.681	2.45		2.450	1	1
BCAL3054	6,7-dimethyl-8-ribityllumazine synthase	1.118	1.249	1.182	1.242	1.118	1.178	0.988	1.108	1.046	0	0

BCAL3055	probable N utilization substance protein B	0.75	0.704	0.727	0.75	1.471	1.050	1.01	0.987	0.998	0	0
BCAL3111	conserved hypothetical protein		0.917	0.917		0.656	0.656		0.675	0.675	0	-1
BCAL3113 (gi118651602)	Phosphomannomutase [*Burkholderia cenocepacia* MC0-3]		0.884	0.884		0.984	0.984		1.031	1.031	0	0
BCAL3115 (gi117991085)	glycosyl transferase, family 2 [*Burkholderia phytofirmans* PsJN]		1.285	1.285		1.545	1.545		1.358	1.358	0	1
BCAL3136 (gi118656806)	apaH, bis(5'nucleosyl)-tetraphosphatase, ApaH [*Burkholderia multivorans* ATCC 17616]		1.414	1.414		2.322	2.322		1.543	1.543	0	1
BCAL3142	conserved hypothetical protein		0.664	0.664		0.672	0.672		1.003	1.003	-1	0
BCAL3146	60 kDa chaperonin 1	0.888	0.905	0.896	1.224	1.05	1.134	1.052	0.942	0.995	0	0
BCAL3147	10 kDa chaperonin 1	1.062	1.009	1.035	1.471	0.772	1.066	1.034	0.791	0.904	0	0
BCAL3166	putative lipoprotein	0.84	1.035	0.932	0.99	1.121	1.053	0.983	1.13	1.054	0	0
BCAL3178	LysR family regulatory protein	0.792	0.516	0.639	0.709	0.588	0.646	0.827	0.7	0.761	-1	-1
BCAL3183	putative hydrolase	1.296	1.277	1.286	1.824	1.335	1.560	1.121	1.152	1.136	0	1
BCAL3186	conserved hypothetical protein	0.4		0.400	1.108		1.108	0.853		0.853	-1	0
BCAL3191	putative glutaryl-CoA dehydrogenase	0.664	0.663	0.663	0.744	0.749	0.746	1.013	0.898	0.954	-1	0
BCAL3197	serine hydroxymethyltransferase	1.003	0.433	0.659	1.088	0.803	0.935	1.034	0.984	1.009	-1	0
BCAL3204	putative OmpA family lipoprotein	0.876	0.977	0.925	0.629	0.825	0.720	0.913	1.191	1.043	0	0
BCAL3205	putative exported protein	0.915	0.876	0.895	1.02	0.994	1.007	1.01	1.004	1.007	0	0
BCAL3220 (gi115285990)	beta-ketoacyl synthase [*Burkholderia cepacia* AMMD]	0.587	1.251	0.857	0.209	1.692	0.595	0.815	2.471	1.419	0	-1
BCAL3244 (gi83654978)	possible glycosyltransferase WbpX, putative [*Burkholderia thailandensis* E264]											
BCAL3270	putative DnaK chaperone protein	1.108	1.006	1.056	1.161	0.873	1.007	0.994	0.789	0.886	0	0
BCAL3272	putative heat shock protein	1.133	1.226	1.179	1.048	1.062	1.055	1.07	0.927	0.996	0	0
BCAL3299	peroxidase/catalase KatB	1.282	1.222	1.252	1.533	1.51	1.521	1.111	1.054	1.082	0	1
BCAL3316 (gi117985821)	protein of unknown function DUF330 [*Burkholderia phymatum* STM815]	0.624		0.624	0.375		0.375	0.872		0.872	-1	-1
BCAL3332	aminopeptidase P		0.683	0.683		0.546	0.546		0.694	0.694	0	-1
BCAL3347	30S ribosomal protein S9	0.92	0.711	0.809	0.829	0.785	0.807	0.652	0.558	0.603	0	0
BCAL3358	periplasmic glutamate/aspartate-binding protein	1.213	1.18	1.196	1.51	1.148	1.317	1.003	0.85	0.923	0	0
BCAL3359	putative glutamate dehydrogenase	1.221	0.844	1.015	1.194	0.878	1.024	1.048	1.312	1.173	0	0
BCAL3368 (gi117980776)	transcriptional regulator, RpiR family [*Burkholderia phymatum* STM815]	1.447		1.447	1.11		1.110	1.188		1.188	0	0
BCAL3370	gamma-glutamyl phosphate reductase		0.73	0.730		0.564	0.564		0.848	0.848	0	-1
BCAL3377	putative outer membrane protein		4.041	4.041		3.388	3.388		1.71	1.710	1	1
BCAL3378	ferric uptake regulator	0.556	1.048	0.763	0.626	0.96	0.775	0.657	0.617	0.637	0	0
BCAL3388	glyceraldehyde 3-phosphate dehydrogenase 1	1.198	1.208	1.203	1.377	1.382	1.379	1.213	1.206	1.209	0	0
BCAL3389	transketolase 1	0.743		0.743	0.903		0.903	0.943		0.943	0	0
BCAL3402	short-chain dehydrogenase/reductase SDR											

(gi118641093)	[*Burkholderia ambifaria* MC40-6]											
BCAL3425	putative sugar kinase 3		0.555	0.555		1.045	1.045		0.713	0.713	-1	0
BCAL3426	putative lipoprotein		1.17	1.170		0.716	0.716		1.167	1.167	0	0
BCAL3427	histone H1-like protein	1.434	1.092	1.251	1.964	0.791	1.246	0.575	0.294	0.411	0	0
BCAL3429	putative ribonucleoside reductase	0.618	0.797	0.702	0.477	0.796	0.616	0.874	0.76	0.815	0	-1
BCAL3436	prolyl-tRNA synthetase		0.923	0.923		0.665	0.665		0.86	0.860	0	-1
BCAL3467	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate		1.281	1.281		1.54	1.540		1.094	1.094	0	1
BCAL3489 (gi76583601)	conserved hypothetical protein [*Burkholderia pseudomallei* 1710b]		1.446	1.446		1.804	1.804		1.875	1.875	0	1
BCAL3507	flagellar FliL protein		1.087	1.087		1.094	1.094		1.131	1.131	0	0
BCAL3523	general secretory pathway protein G		1.334	1.334		0.967	0.967		1.496	1.496	0	0
BCAL3529	putative cobalamin synthesis protein/P47K	1.459		1.459	1.504		1.504	1.502		1.502	0	1
BCAL3530	DNA-binding protein HU-alpha	1.014	1.065	1.039	1.008	0.74	0.864	0.956	0.65	0.788	0	0
BCAM0006	conserved hypothetical protein	0.609		0.609	0.672		0.672	0.807		0.807	-1	0
BCAM0026	putative siderophore-interacting protein	0.308		0.308	0.327		0.327	0.871		0.871	-1	-1
BCAM0058	3-oxoadipate CoA-transferase subunit A		1.168	1.168		1.784	1.784		2.035	2.035	0	1
BCAM0060 (gi52421938)	3-carboxy-cis,cis-muconate cycloisomerase [*Burkholderia mallei* ATCC 23344]	0.722		0.722	0.3		0.300	1.151		1.151	0	-1
BCAM0149 (gi77971729)	phospholipase D/Transphosphatidylase [*Burkholderia* sp. 383]	1.052	0.925	0.986	0.9	0.851	0.875	0.873	0.901	0.887	0	0
BCAM0173	ATP-independent RNA helicase		0.569	0.569		0.709	0.709		1.427	1.427	-1	0
BCAM0175 (gi77971682)	malate dehydrogenase (acceptor) [*Burkholderia* sp. 383]											
BCAM0184	lectin	0.387	0.51	0.444	0.351	0.549	0.439	1.004	1.222	1.108	-1	-1
BCAM0186	lectin	0.851	0.432	0.606	0.727	0.39	0.532	0.999	0.764	0.874	-1	-1
BCAM0276 (gi115285786)	UspA domain protein [*Burkholderia cepacia* AMMD]	1.048	0.745	0.884	0.892	0.652	0.763	0.782	0.689	0.734	0	0
BCAM0297 (gi115283858)	acetoacetyl-CoA reductase [*Burkholderia cepacia* AMMD]											
BCAM0313	putative exported protein											
BCAM0357	LysR family regulatory protein											
BCAM0502	conserved hypothetical protein											
BCAM0504	CsbD-like protein	1.105	1.026	1.065	0.968	0.681	0.812	0.798	0.566	0.672	0	0
BCAM0505	putative membrane-attached protein	1.104	0.977	1.039	1.046	0.863	0.950	0.793	0.806	0.799	0	0
BCAM0507	CsbD-like protein	0.897	0.887	0.892	0.732	1.069	0.885	0.741	1.019	0.869	0	0
BCAM0589	conserved hypothetical protein		3.914	3.914		3.942	3.942		2.907	2.907	1	1
BCAM0600	conserved hypothetical protein		0.89	0.890		0.906	0.906		1.052	1.052	0	0
BCAM0602 (gi134135913)	molecular chaperone-like protein [*Burkholderia vietnamiensis* G4]	1.534		1.534	1.387		1.387	0.711		0.711	1	0
BCAM0630	putative dehydrogenase											

BCAM0684	putative oxidoreductase		1.55	1.550		0.846	0.846		0.854	0.854	1	0
BCAM0759 (gi134132844)	extracellular solute-binding protein, family 3 [*Burkholderia vietnamiensis* G4]	0.783		0.783	0.459		0.459	1.135		1.135	0	-1
BCAM0767 (gi117992836)	ABC transporter related [*Burkholderia phytofirmans* PsJN]	0.624		0.624	0.469		0.469	1.005		1.005	-1	-1
BCAM0795 (gi117992171)	transcriptional regulator, MarR family [*Burkholderia phytofirmans* PsJN]											
BCAM0811	putative aromatic oxygenase		0.402	0.402		0.909	0.909		1.308	1.308	-1	0
BCAM0829	putative riboflavin synthase alpha chain	1.564	0.757	1.088	1.855	1.585	1.715	2.207	1.121	1.573	0	1
BCAM0832 (gi105893144)	Dyp-type peroxidase [*Burkholderia cenocepacia* AU 1054]	1.387	1.39	1.388	1.825	1.384	1.589	1.045	0.97	1.007	0	1
BCAM0833 (gi118660931)	OsmC family protein [*Burkholderia multivorans* ATCC 17616]											
BCAM0844	conserved hypothetical protein	1.096	0.993	1.043	0.776	0.808	0.792	0.876	1.017	0.944	0	0
BCAM0910	putative geranyltranstransferase	0.79		0.790	0.501		0.501	1.013		1.013	0	-1
BCAM0915	30S ribosomal protein S21 2	1.315	1.056	1.178	1.406	1.134	1.263	1.161	1.072	1.116	0	0
BCAM0916	conserved hypothetical protein	0.926	1.13	1.023	1.054	1.235	1.141	0.983	1.279	1.121	0	0
BCAM0917	putative DNA primase	1.455		1.455	1.616		1.616	1.818		1.818	0	1
BCAM0953	extracellular solute-binding protein	0.971	0.458	0.667	1.032	1.696	1.323	0.9	1.055	0.974	-1	0
BCAM0961	aconitate hydratase	1.106	1.055	1.080	1.245	0.733	0.955	1.36	1.233	1.295	0	0
BCAM0963	putative exported protein	0.934	0.985	0.959	1.238	1.202	1.220	1.342	1.442	1.391	0	0
BCAM0972	citrate synthase 1	1.12	0.972	1.043	0.508	0.396	0.449	0.44	0.389	0.414	0	-1
BCAM0986	aspartate-semialdehyde dehydrogenase	1.038	1.107	1.072	1.618	1.7	1.658	1.362	1.639	1.494	0	1
BCAM0987	flagellar hook protein 2 FlgE2											
BCAM0988	putative exported protein		1.712	1.712		2.279	2.279		1	1.000	1	1
BCAM1003	putative epimerase											
BCAM1012	putative histone-like protein	1.198	1.038	1.115	1.371	0.899	1.110	0.888	0.775	0.830	0	0
BCAM1015	putative porin		0.85	0.850		0.998	0.998		1.182	1.182	0	0
BCAM1021	protein FdhE homologue		0.529	0.529		0.659	0.659		0.48	0.480	-1	-1
BCAM1062	hypothetical phage protein											
BCAM1111	ornithine decarboxylase		1.102	1.102		1.318	1.318		1.115	1.115	0	0
BCAM1123 (gi91692859)	hypothetical protein [*Burkholderia xenovorans* LB400]											
BCAM1189 (gi115283494)	transcriptional regulator, LysR family [*Burkholderia cepacia* AMMD]	0.735		0.735	0.489		0.489	0.998		0.998	0	-1
BCAM1204	alanine racemase, catabolic		0.855	0.855		0.395	0.395		0.835	0.835	0	-1
BCAM1234	conserved hypothetical protein											
BCAM1250 (gi77964942)	acetyl-CoA hydrolase/transferase [*Burkholderia* sp. 383]	0.907		0.907	0.581		0.581	1.13		1.130	0	-1
BCAM1291	L-asparaginase		0.915	0.915		0.652	0.652		0.688	0.688	0	-1
BCAM1304	conserved hypothetical protein	0.541		0.541	0.433		0.433	1.13		1.130	-1	-1

BCAM1351	putative regulatory protein		1.036	1.036		1.054	1.054		0.656	0.656	0	0
BCAM1380 (gi118650657)	transcriptional regulator, GntR family [*Burkholderia cenocepacia* MC0-3]											
BCAM1407	DJ-1/PfpI family protein	0.776	0.997	0.880	0.609	0.688	0.647	0.718	0.765	0.741	0	-1
BCAM1411	putative short-chain dehydrogenase		0.715	0.715		0.626	0.626		1.06	1.060	0	-1
BCAM1412 (gi105895814)	conserved hypothetical protein [*Burkholderia cenocepacia* AU 1054]	0.384		0.384	0.233		0.233	0.655		0.655	-1	-1
BCAM1443	putative exported protein	0.681	1.022	0.834	0.525	0.952	0.707	0.545	0.849	0.680	0	0
BCAM1464 (gi116650430)	putative cytoplasmic protein [*Burkholderia cenocepacia* HI2424]	0.918	1.254	1.073	0.868	1.359	1.086	0.923	1.485	1.171	0	0
BCAM1481	conserved hypothetical protein	0.842	1.011	0.923	0.759	1.035	0.886	0.962	1.073	1.016	0	0
BCAM1491 (gi116650457)	conserved hypothetical protein [*Burkholderia cenocepacia* HI2424]		1.864	1.864		1.768	1.768		0.605	0.605	1	1
BCAM1538	putative dehydrogenase, monooxygenase subunit	0.906	1.402	1.127	1.406	1.044	1.212	1.21	0.998	1.099	0	0
BCAM1542	putative aldehyde dehydrogenase	0.911	0.879	0.895	1.138	0.99	1.061	1.389	1.362	1.375	0	0
BCAM1553	putative dehydrogenase, molybdenum-binding		1.635	1.635		1.139	1.139		1.125	1.125	1	0
BCAM1570	alcohol dehydrogenase	0.921	0.826	0.872	1.041	0.761	0.890	0.93	0.828	0.878	0	0
BCAM1581	phosphoenolpyruvate carboxykinase [GTP]	0.892	0.892	0.892	0.678	0.83	0.750	0.877	0.727	0.798	0	0
BCAM1619	putative DNA-binding cold-shock protein		0			1.892	1.892		0			1
BCAM1654 (gi77969268)	Short-chain dehydrogenase/reductase SDR [*Burkholderia* sp. 383]											
BCAM1669	putative exported protein	0.767	0.973	0.864	1.189	0.274	0.571	0.916	1.105	1.006	0	-1
BCAM1679 (gi117985553)	protein of unknown function DUF472 [*Burkholderia phymatum* STM815]											
BCAM1700 (gi117993315)	GCN5-related N-acetyltransferase [*Burkholderia phytofirmans* PsJN]		1.793	1.793		1.948	1.948		1.332	1.332	1	1
BCAM1744	serine peptidase, family S9											
BCAM1800	conserved hypothetical protein		1.555	1.555		1.235	1.235		1.641	1.641	1	0
BCAM1824	conserved hypothetical protein		1.605	1.605		1.052	1.052		1.469	1.469	1	0
BCAM1857	conserved hypothetical protein											
BCAM1919 (gi118648785)	conserved hypothetical protein [*Burkholderia cenocepacia* MC0-3]	0.848		0.848	0.553		0.553	0.751		0.751	0	-1
BCAM1926	conserved hypothetical protein	0.83	0.517	0.655	0.989	0.774	0.875	0.837	0.462	0.622	-1	0
BCAM1931	putative porin											
BCAM2001 (gi118650319)	short-chain dehydrogenase/reductase SDR [*Burkholderia cenocepacia* MC0-3]	1.067		1.067	0.643		0.643	1.14		1.140	0	-1
BCAM2006	putative aspartate carbomyltransferase		0.467	0.467		1.626	1.626		0.957	0.957	-1	1
BCAM2068	conserved hypothetical protein	0.87		0.870	0.564		0.564	0.79		0.790	0	-1
BCAM2081	conserved hypothetical protein	1.565		1.565	3.032		3.032	1.198		1.198	1	1
BCAM2143	cable pilus associated adhesin protein	0.535	0.603	0.568	0.349	0.578	0.449	0.614	0.764	0.685	-1	-1
BCAM2159	putative exported protein	1.313	1.152	1.230	1.211	0.892	1.039	0.969	0.822	0.892	0	0

BCAM2167	conserved hypothetical protein	1.167	1.087	1.126	1.149	1.271	1.208	0.9	1.083	0.987	0	0
BCAM2192	enoyl-CoA hydratase/isomerase family protein	1.317	1.271	1.294	2.137	1.629	1.866	1.21	0.972	1.084	0	1
BCAM2195	putative AMP-binding enzyme	0.537	0.644	0.588	0.683	0.728	0.705	1.032	1.004	1.018	-1	0
BCAM2234 (gi76582946)	salicylate biosynthesis protein [*Burkholderia pseudomallei* 1710b]	0.949		0.949	0.48		0.480	1.223		1.223	0	-1
BCAM2307	zinc metalloprotease ZmpB	0.251	0.328	0.287	0.296	0.362	0.327	0.815	0.804	0.809	-1	-1
BCAM2308	putative leucyl aminopeptidase precursor		0.265	0.265		0.291	0.291		0.816	0.816	-1	-1
BCAM2310 (gi52422765)	putative transporter [*Burkholderia mallei* ATCC 23344]		0.71	0.710		0.565	0.565		0.26	0.260	0	-1
BCAM2378	putative Xaa-Pro dipeptidyl-peptidase											
BCAM2407 (gi117982799)	major facilitator superfamily MFS_1 [*Burkholderia phymatum* STM815]											
BCAM2456 (gi118660218)	peptidoglycan glycosyltransferase [*Burkholderia multivorans* ATCC 17616]	0.999		0.999	0.606		0.606	0.93		0.930	0	-1
BCAM2463	putative membrane protein											
BCAM2468	putative aldehyde dehydrogenase family protein											
BCAM2478	serine-carboxyl peptidase		0.856	0.856		0.476	0.476		1.207	1.207	0	-1
BCAM2480 (gi52423083)	transcriptional regulator, AsnC family [*Burkholderia mallei* ATCC 23344]											
BCAM2556	putative purine nucleoside permease	0.9	0.811	0.854	0.837	0.572	0.692	1.052	0.871	0.957	0	0
BCAM2618	putative periplasmic	1.122	1.108	1.115	1.409	1.154	1.275	1.035	0.952	0.993	0	0
BCAM2671	putative low-specificity L-threonine aldolase	1.387	0.94	1.142	1.951	1.11	1.472	1.181	1.087	1.133	0	0
BCAM2702	2-methylcitrate synthase	0.904	0.901	0.902	0.825	0.849	0.837	0.801	0.838	0.819	0	0
BCAM2707	putative FAA-hydrolase family protein	1.062	1.423	1.229	1.496	1.521	1.508	1.207	1.521	1.355	0	1
BCAM2755	putative exported protein	0.873	0.941	0.906	0.945	0.751	0.842	0.749	0.743	0.746	0	0
BCAM2783 (gi117988745)	glycoside hydrolase, family 28 [*Burkholderia phytofirmans* PsJN]		1.09	1.090		1.83	1.830		2.021	2.021	0	1
BCAM2821	malate synthase G	0.98	0.643	0.794	0.686	0.637	0.661	0.843	0.83	0.836	0	-1
BCAM2827	putative exported protein	1.092	1.032	1.062	1.024	1.11	1.066	1.083	1.006	1.044	0	0
BCAS0010 (gi77965393)	protein of unknown function DUF6, transmembrane [*Burkholderia* sp. 383]	1.085	0.91	0.994	1.033	0.934	0.982	1.065	0.888	0.972	0	0
BCAS0013	putative molybdenum transport protein	0.867	2.937	1.596	0.838	2.451	1.433	1.041	3.243	1.837	1	0
BCAS0070 (gi118660709)	histidine kinase [*Burkholderia multivorans* ATCC 17616]											
BCAS0151	hypothetical protein											
BCAS0190	putative H-NS family DNA-binding protein	1.051	1.022	1.036	0.939	0.919	0.929	0.736	0.899	0.813	0	0
BCAS0292	conserved hypothetical protein AidA'		0.262	0.262		0.177	0.177		0.735	0.735	-1	-1
BCAS0293	nematocidal protein AidA	0.023	0.06	0.037	0.044	0.01	0.021	0.566	0.633	0.599	-1	-1
BCAS0334 (gi134132307)	response regulator receiver protein [*Burkholderia vietnamiensis* G4]											
BCAS0382	transcriptional regulator, AsnC family	0.846		0.846	0.417		0.417	0.855		0.855	0	-1

(gi117994159)	[*Burkholderia phytofirmans* PsJN]											
BCAS0407	hypothetical protein	0.613		0.613	0.812		0.812	0.852		0.852	-1	0
BCAS0409	zinc metalloprotease ZmpA	0.799	0.672	0.733	0.92	0.743	0.827	1.02	0.967	0.993	0	0
BCAS0421 (gi76580611)	ABC transporter, ATP-binding protein [*Burkholderia pseudomallei* 1710b]											
BCAS0455	putative CopG family protein		1.454	1.454		1.65	1.650		1.818	1.818	0	1
BCAS0516 (gi115281897)	hypothetical protein [*Burkholderia cepacia* AMMD]											
BCAS0609	putative electron transfer		0.616	0.616		0.609	0.609		0.948	0.948	-1	-1
BCAS0637	60 kDa chaperonin 3	0.819	0.781	0.800	1.036	0.945	0.989	1.119	1.133	1.126	0	0
BCAS0638	10 kDa chaperonin 3	1.233	0.868	1.035	2.31	1.056	1.562	1.359	1.017	1.176	0	1
BCAS0666	putative ankyrin-repeat exported protein		1.798	1.798		1.851	1.851		1.228	1.228	1	1
BCAS0708 (gi91686888)	hypothetical protein [*Burkholderia xenovorans* LB400]		0.636	0.636		0.697	0.697		0.755	0.755	-1	0
BCAS0730 (gi77964328)	Na+ dependent nucleoside transporter [*Burkholderia* sp. 383]	0.902		0.902	0.619		0.619	1.013		1.013	0	-1
BCAS0739	putative acetyl-CoA synthetase											
BCAS0747 (gi117980068)	SMC protein-like [*Burkholderia phymatum* STM815]	0.61	1.107	0.822	0.312	1.255	0.626	0.746	1.285	0.979	0	-1
BCAS0765 (gi77969929)												
Holden antidrug resist	hydrophobe/amphiphile efflux pump, HAE1 family [*Burkholderia* sp. 383]											
BCAS0773	putative exported protein	0.922	0.98	0.951	1.197	1.301	1.248	0.752	1.236	0.964	0	0
rmlC (gi52210708)	dTDP-6-deoxy-D-glucose-3,5 epimerase [*Burkholderia pseudomallei* K96243]		1.503	1.503		1.343	1.343		1.156	1.156	1	0
wbiF (gi134134232)	glycosyl transferase, family 2 [*Burkholderia vietnamiensis* G4]											
xdhA (gi115283038)	transcriptional regulator, LysR family [*Burkholderia cepacia* AMMD]											

Acknowledgements

At first, I would like to express my gratitude to my supervisor, Prof. Dr. Leo Eberl, for his steady guidance, his scientific mentoring, for providing me with the opportunity to participate in challenging research projects, and his constant willingness to engage in valuable discussions about the research of my work.

I would like to thank Prof. Dr. Kathrin Riedel for her great engagement and contribution to the research of this work, for fruitful discussions and ideas, her continuous help, and for the willingness to serve as a member of my examination committee.

Prof. Dr. Jakob Pernthaler I would like to thank for kindly serving as a second faculty member of my examination committee.

Prof. Dr. Pamela Sokol from the University of Calgary I would like to thank for the good cooperation concerning the analysis of the CepR2 regulon and for the external review of this thesis.

I would like to thank Dr. Claudio Aguilar for his continuous interest in my work and steady support, for his good ideas with the set-up of experiments, for generously spending his time reading and revising my work, and for his valuable feedback.

I would like to thank Dr. Andrea Sass and Prof. Dr. Eshwar Mahenthiralingam from the School of Bioscience at Cardiff University for the performance of the microarray analysis at their facilities. I am grateful for what I learned in their laboratory and for their hospitality in Cardiff.

I would like to thank Alex for his work and exhausless patience with the lectins. Urs Ziegler I would like that thank for his support concerning the microscopy of the lectins and for providing the University facilities of the Functional Genomic Center.

I thank all of members of the Eberl group war their support, especially “the girls” for the warm welcome in Zürich, Susanne for her help with the BIOLOG data, Aurelien for advices and discussions, and Stephan for his help and a great time with the student courses.

At last, I offer my regards to all of those who supported me in any respect during the completion of this thesis. Melanie, I have to especially thank you for your advice and your encouragement over the years, Anna and Steffi, Jutta and Sophie, Nadine, Mali, Iveta and our “international hiking group”, thanks for your numerous motivating words and the great distractions. Finally, a big thank you to my family for their support throughout the years. Gilla, Dieter, Inga and Sönke, I would like to thank you for your continuous faith in me, your criticism, and your moral support and advice – ich danke Euch.

Silja Inhülsen

Forchstrasse 284, 8008 Zürich, Switzerland
Email: sinhuelsen@botinst.uzh.ch
Date and place of birth: January 9th, 1981 in Hannover, Germany

EDUCATION AND EXPERIENCE:

2007 - 2011: *PhD student* (University of Zurich) and *student* of the Life Science Zurich Graduate School "Microbiology and Immunology" (ETH Zurich and University of Zurich, Switzerland)

2006 - 2007: *Editoral intern* and *Science journalist* at Spektrum Publishing House, (Holtzbrinck/ Elsevier), Heidelberg, Germany

2002 - 2006: *Diploma student*, Eberhard-Karls-University Tübingen, Germany, with Microbiology (major subject), Biochemistry and Parasitology (minor subjects)
Thesis in the Department of Microbial Genetics, Prof. Dr. F. Goetz and Prof. Dr. W. Vollmer (2006)
Thesis: *Adr* encodes for a Murein-O-Acetyltransferase in *Streptococcus pneumoniae.*

2002 - 2004: *Graduate assistant*, Max-Planck-Institute (MPI) for Biological Cybernetics and MPI for Developmental Biology, Tübingen, Germany

2001 – 2002: *Graduate assistant*, Institute of Tropical Medicine, Eberhard-Karls-University, Tübingen, Germany

August, 2002: Diving Certificate, Marine Biology Course, HYDRA Institute, Elba, Italy

2000: Qualification for university entrance (Abitur) at Bismarckschule Hannover Gymnasium, Hannover, Germany

1997 – 1998: Senior year at Pulaski High School, Wisconsin, USA